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CYTOLOGICAL STUDIES ON GLOBI IN LEPROSY *

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For over 50 years the nature of large globular masses of bacilli called "globi" has been in question. Despite the fact that they are among the most conspicuous objects observed in the lesions of lepromatous leprosy the controversy about them started by Unna and Hansen is still unsettled. Unna and his associates claimed that the bacilli, arranged in globi, are located mainly extracellularly, especially within lymph spaces, while Hansen, Neisser and others contended that the globi are mainly intracellular accumulations of bacilli. The literature to 1911 has been well summarized by Gurd.[1] In 1934 Denney,[2] at that time Director of the U. S. Marine Hospital (Leprosarium) at Carville, said that although they are "of perhaps the greatest diagnostic importance" their true nature remains undetermined. This was a year after he and Eddy [3] had presented a paper on the subject. In the discussion of this paper many ideas were expressed. Duval remarked that the view held by Unna prevailed until Gurd showed that the "globi" are not actually within the lymphatics but in large endothelial cells (the so-called 'lepra' cell) of the lesion. Wade, in discussing the question with European dermatologists interested in leprosy, said that he had to agree that "such masses may occur in lymph spaces" but he concurred with Denney and Duval that normally they are present in the cells mentioned. It appears,

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therefore, that in Europe the conception of Unna was held as late as 1934, while in this country the majority opinion was that the globi are essentially intracellular formations. It was in an attempt to secure more information that the study reported in this paper was undertaken.

I. Material

Data concerning the principal tissues examined are given in Table I. Colonel S. Simmons, when in Panama, obtained Case 1 from Dr. Kurevitsch. My associate, Dr. L. F. Heimburger, collected Cases 2–7 at the U. S. Marine Hospital (Carville) with the permission of Dr. O. E. Denney. He also contributed Cases 8–12 which he had secured at the Cheeloo Leprosarium in China. Cases 13–20 were collected by me at the Insular Leprosarium in Puerto Rico while I was working in the nearby School of Tropical Medicine under invitation from the Director, Dr. G. W. Bachman. Of these, biopsy in Cases 16–18 was done by Dr. J. Noya Benitez and in Cases 19–20 by Dr. F. Hernandez-Morales. Cases 21–29 were autopsies, the preparations from which were placed at my disposal, during a visit to the U. S. Marine Hospital, by Dr. H. E. Hasseltine, Director, and Dr. Sam H. Black, pathologist. The latter gave me blocks of tissue of special interest for detailed study in St. Louis.

Perspective was gained by examination of biopsies of 10 cases of tuberculoid leprosy in children, sent from the Virgin Islands by Dr. James Knott, because in this type of leprosy globi are not ordinarily found, and it is important to contrast the kinds of lesion in which they do and do not develop. Opportunity to study globi in water buffalo leprosy was afforded by a series of formalin-fixed tissues provided by Dr. H. W. Wade who secured them from Dr. L. W. M. Lobel. Dr. A. Zeissig, acting for Dr. W. A. Hagan, donated tissues from Johne's disease and Dr. Margaret Smith material from human tuberculosis.

To all of these individuals I wish to express my thanks.

II. Technic

Differences in the bacilli, or in the lesions, owing to variations in technic, are not likely to occur. In an experiment repeated three times it was not possible to detect any significant differ-

TABLE I

Clinical History

Case number	Age	Sex	Race	Type of leprosy *	Duration of lesions	Region of specimen	Kind of treatment	Origin of specimen	Autopsy or biopsy
	yrs.				*yrs.*				
1	70	M	Negro	Cutaneous C_3	10	Thigh	Chaulmoogra 10+ yrs.	Panama	Biopsy
2	42	M	Mexican		14			Carville	Autopsy F. S. 1015
3	42	M	Mexican	C_2N_1	4	Ear lobes	None	Carville	Biopsy P. A. 1049
4	30	M	White	C_2N_1	10	Forearm	Routine Chaulmoogra	Carville	Biopsy W. M. 619
5	36	M	Mexican	C_2N_2	5	Forehead	None	Carville	Biopsy F. H. 971
6	35	M	White	C_2N_1	19	Nape of neck	Routine Chaulmoogra	Carville	Biopsy F. V. 514
7	30	M	Mexican	C_2N_1	8	Face and forearm	None	Carville	Biopsy J. P. 1050
8	16	M	Chinese	C_1	2	Upper arm	None	China	Biopsy G 34981
9	19	M	Chinese	C_1N_1	5	Forearm	Irregular	China	Biopsy G 35846

TABLE I (*Continued*)

Case number	Age	Sex	Race	Type of leprosy	Duration of lesions	Region of specimen	Kind of treatment	Origin of specimen	Autopsy or biopsy
	yrs.				*yrs.*				
10	31	M	Chinese	C_2	5	Forearm	None	China	Biopsy E 72466
11	45	M	Chinese	N_1	1	Upper arm	None	China	Biopsy G 40015
12	30	M	Chinese	N_2	3	Lower leg	None	China	Biopsy G 55760
13	44	F	Negro Indian	Cutaneous nodular	7	Forearm	Chaulmoogra oil	Puerto Rico	Biopsy C. M.
14	17	M	Puerto Rican	Cutaneous nodular	9	Forearm	Chaulmoogra + methylene blue	Puerto Rico	Biopsy R. R.
15	12	M	Negro	Cutaneous nodular	4	Ear crest and lobule	Chaulmoogra	Puerto Rico	Biopsy R. P.
16	22	M	Puerto Rican	Cutaneous nodular	9	Ear	Chaulmoogra orally, esters injected	Puerto Rico	Biopsy P. V.
17	25	M	Puerto Rican	Cutaneous nodular	18	Forearm	Chaulmoogra	Puerto Rico	Biopsy E. B. G.
18		M	Puerto Rican	Cutaneous nodular		Alae nasi		Puerto Rico	Biopsy E. U. C.
19	23	M	Puerto Rican	Cutaneous nodular	9	Forearm	Chaulmoogra orally, esters injected	Puerto Rico	Biopsy G. R.
20	35	M	Puerto Rican	Cutaneous nodular	16	Forearm	Chaulmoogra	Puerto Rico	Biopsy P. J. G.

21	42	M	White	Mixed	10	Several	Chaulmoogra 4 years	Carville	Autopsy No. 723
22	62	M	White	Mixed, cutaneous predominating	21	Several	Chaulmoogra 8 yrs. Fowler's sol. 1 yr.	Carville	Autopsy No. 708
23	64	F	White	Mixed, cutaneous predominating	25	Several	Chaulmoogra 16 yrs.	Carville	Autopsy No. 552
24	29	M	White	Mixed	12	Several	Chaulmoogra 2½ yrs.	Carville	Autopsy No. 880
25	53	M	White	Mixed, cutaneous predominating	17	Several	Chaulmoogra 6 months	Carville	Autopsy No. 1057
26	54	M	Chinese	Mixed	About 21	Several	Chaulmoogra and Fowler's sol. 8 yrs.	Carville	Autopsy No. 165
27	40	M	Negro	Mixed	7	Several	Chaulmoogra 1 yr.	Carville	Autopsy No. 931
28	42	M	White	Mixed	About 21	Several	Chaulmoogra 1 yr.	Carville	Autopsy No. 775
29	25	M	White	Mixed	About 17	Several	Chaulmoogra 3 yrs. Mercurochrome 1 mo.	Carville	Autopsy No. 339

* The diagnoses of clinical types of leprosy are listed as given by the clinicians kindly supplying materials. Some employed Leonard Wood Memorial Conference letters (see *Inter. J. Leprosy*, 1934, **2**, 329–356), while others did not. To attempt to bring all under a single method of classification would introduce errors.

ence in the bacilli depending on whether the tissues were fixed in:

- Regaud's fluid
- Zenker less acetic acid
- Zenker + 5 per cent acetic acid
- Zenker + 10 per cent acetic acid
- Zenker less acetic acid + 10 per cent formalin
- 95 per cent alcohol + 10 per cent formalin
- 10 per cent formalin alone

Osmic acid mixtures (Bensley's A.O.B., Altmann's fluid and 1 per cent and 2 per cent osmic acid) gave good preservation of the bacilli but penetrated so poorly as to limit their usefulness. Saturated aqueous mercuric chloride plus 5 per cent acetic acid and saturated 80 per cent alcoholic mercuric chloride plus 5 per cent acetic acid gave good results. Simple fixation in 80 per cent alcohol, which is usually regarded as the best fixative for acid-fast bacilli, gave beautiful preparations of the bacilli at the expense of the tissues, which were poorly preserved. The freezing and drying technic [4] is recommended for leprosy bacilli but it is a slow method and is not feasible except in a laboratory equipped with a cryostat.

Slight delay in the fixation of tissues after their removal did not lead to alterations in the bacilli. For example, a nodule was excised, part of it immediately fixed in Regaud's fluid, and the remainder fixed in the same way after it had been kept in a Petri dish with cotton moistened with physiological saline solution in the ice box for 16½ hours and after this at room temperature for 11½ hours. The bacilli in stained sections of both were compared. They appeared if anything to be better preserved and stained after the delayed fixation but this may be, as in the case of pure alcohol fixation, because the tissue components suffer and relative to them the bacilli are in excellent condition.

When, however, the surface of the tissue was allowed to dry before fixation the dehydration caused profound alterations in the globi and other structures. Pinching of the tissues with forceps, and to a less extent cutting with scissors, also produced confusing modifications and for this reason the tissues were lifted and cut with a wet razor blade.

The Ziehl-Neelsen method of staining acid-fast bacteria with

fuchsin was employed except that hematoxylin was used as a counterstain in place of methylene blue because of its greater permanence. Precipitation of the fuchsin on the sections was prevented by covering them with a layer of filter paper before application and heating of the stain. The "new fuchsin" recommended by Fite [5] was tried but improvement over results obtained with other fuchsins was not great. Carpano's [6] technic for staining the bacilli dark violet in a yellow background gave beautiful preparations. For the coloration of both bacilli and fat in the same preparations the following method was used.

1. Cut frozen sections 10 to 15 μ thick.
2. Stain sections on the slide with carbol fuchsin with the aid of heat for 20 minutes.
3. Differentiate with acid alcohol until the sections are a dark pink.
4. Wash in distilled water.
5. Counterstain with Harris' hematoxylin for 10 minutes.
6. Differentiate in acid alcohol.
7. Neutralize with very dilute ammonia water. The sections should become blue.
8. Wash well in distilled water.
9. Stain for fat in a saturated solution of Sudan IV (scarlet red) in 70 per cent alcohol for from 1–3 hours.
10. Rinse in 70 per cent alcohol.
11. Rinse in distilled water.
12. Mount sections on a slide with glycerin and seal around the coverslip with a beeswax-balsam compound.

In order to make sure that no bacilli were missed, particularly non-acid-fast ones, Giemsa's stain was frequently applied to the sections. Except when undue pressure was exerted on the coverglasses, no signs were found of the displacement of bacilli in the globi so that the method advised by Lobel [7] (in water buffalo leprosy) to hold them in place was not used.

III. Observations

The ideal way would have been to study the sequence of changes in the development of globi and to closely relate the changes observed to the evolution of the lesions. This, however,

could not be done owing to the impossibility of collecting specimens in the latent period and the lack of clinical data as to the age of the particular nodules which were biopsied. Data on the duration of the disease in the several cases is not sufficient, since a single patient may show on his arms, for example, nodules of quite different age and structure.

Instead, the approach has been to consider: the distribution of bacilli, whether extracellular or intracellular; the formation of small and of giant globi; the significance of globus formation; and the intracellular behavior of other acid-fast bacteria as compared with that of *Mycobacterium leprae*.

1. Distribution of Bacilli

Sufficient specimens from deep lying tissues, collected at autopsy, were not studied to permit a detailed statement of the distribution of bacilli throughout the whole body. The following remarks relate only to the skin as observed in a large series of biopsies.

Extracellular bacilli must obviously exist in any growing lesion because additional cells could not become involved except by the entry of bacilli which before entry were extracellular. Usually they are difficult to find, but when pressure is exerted on the coverglass bacilli, which were intracellular *in vivo*, may leave the cells in small numbers. Similarly extracellular bacilli, near the edges of sections, are to be interpreted with caution for a certain amount of injury is unavoidable in excising the specimens.

Nerve twigs were observed in some of the sections and a few extracellular bacilli were noted between the fibers, but no attempt was made to gather evidence on Muir's [8] contention that the reservoir of organisms is neural.

Small lymphatic vessels, at the periphery of some of the nodules, exhibited in rare cases sparsely scattered extracellular bacilli. Large lymphatic vessels were not examined in autopsy specimens. Reference will be made later to the sinuses of lymph nodes.

Within the lumens of blood vessels, especially capillaries and venules, extracellular bacilli were noted but they were never numerous and often extremely difficult to find.

Extracellular bacilli were regularly seen in the liquefying centers of lesions where there is much cellular disintegration coupled

with marked invasion by neutrophils. Even in this situation there were no traces of the extracellular development of globi.

Undoubtedly the bacilli are overwhelmingly intracellular. The cells that possess them may be listed in three categories. The first includes those in which the bacilli most commonly occur. Chief among these are the monocytes, sometimes rather loosely referred to as large mononuclear leukocytes, endothelial leukocytes and even as macrophages. Intravascular monocytes laden with bacilli can be studied as individuals and are easily identified.

It is the majority of the extravascular cells, containing simple nuclei, reminiscent of those of typical monocytes, and considerable numbers of bacilli that are most difficult to classify. This is not surprising because there is little uniformity of opinion among cytologists as to the kinds and sources of the cells found in any area of inflammation even though it has been experimentally produced by a known excitant and the stages in its development have been followed from the beginning. In human leprosy we are at a great disadvantage since the lesions, when examined, are of long standing and many of the cells are more distorted by the enormous numbers of bacilli they contain than are similar cells in any other human disease. It would not be so confusing if the swelling were equal, but cells sometimes multinucleated and engorged almost beyond recognition exist side by side with others containing comparatively few bacilli and they are usually all closely pressed together.

Whether the cells with bacilli are monocytes which have invaded the tissue fluids from the blood stream, or monocytes which have developed from extravascular lymphocytes, or histiocytes which have taken up bacilli, or whether they are derived from all three sources may be impossible to determine. The fact that leprologists with one voice remark on the infrequency of cell division in nodules that are enlarging is weak evidence that the increase in cells is occasioned more by cellular immigration than by division of resident tissue cells.

To call the cells engorged with bacilli *epithelioid cells* is misleading. They are only like stratified epithelium in being close together and they are obviously not epithelial. To refer to them as *macrophages*, or "big eaters," is more justified, but they do not as a rule digest the bacilli which they contain. They range all the

way from cells of small size with a single nucleus to giant cells with many nuclei.

In the so-called "foam," Virchow or *lepra cells*, which are often multinucleated, there are many bacilli but the bacilli are in some cases degenerating and there is a good deal of fatty lipoid material. These cells appear to be formed from macrophages but it is not certain that some of them are not derivatives of fibroblasts which are frequently heavily charged with bacilli.

Bacilli commonly occur also in the *endothelial cells* of blood vessels. These differ from the "special endothelial cells" of the liver, spleen and certain other organs in that they are not parts of the reticuloendothelial system.

Neutrophilic leukocytes, which invade the centers of nodules becoming necrotic, almost invariably exhibit bacilli. When, however, they are in the blood stream or in extravascular situations where there is but little necrosis bacilli are not so regularly seen in their cytoplasm.

The second group of cells comprises those that only occasionally contain bacilli. *Epithelial cells* of the epidermis, hair follicles and sweat glands come under this category. Also, bacilli are sometimes observed in small numbers in the peripheral cytoplasm of fat cells. There is some doubt as to whether the endothelial cells of lymphatic vessels and the mesenchymatous cells of the perineurium, epineurium, and endoneurium, in which bacilli are seen, should be listed here or in the first group for which they might qualify had the search been more intensive.

The third group of cells, which are not known to harbor the bacilli in their cytoplasm, requires particular care, for the possibility must be borne in mind that in unusual lesions, complicated in various unexpected ways, they may possess them. I have not encountered any leprosy bacilli in typical *lymphocytes*, either in the blood or extravascularly in the tissue fluid. Others have emphasized their absence in lymphocytes. This is interesting because some leading cytologists believe that monocytes are developed from lymphocytes and monocytes harbor the bacilli more regularly and conspicuously than any other kind of cells.

No bacilli were observed in *plasma cells* of the Marschalko type although such cells are often abundant in the lesions. However plasma cells, as described by Unna, do contain bacilli for Unna

included cells which now go under the name of monocytes in his category of plasma cells.

Russell body cells occur only rarely in cutaneous leprous nodules and when they were seen no bacilli were found within them.

Basophilic leukocytes were not observed within any of the blood vessels cut in sections. It is improbable that they carry bacilli. *Tissue basophils* were fairly numerous in the less dense lesions. No definite bacilli were detected in their cytoplasm but a very small proportion of them contained one, seldom more, fuchsin-stained, acid-resistant droplets which may possibly have been of bacillary origin.

Neither were bacilli noted in blood or tissue *eosinophils* but I am, of course, not ready to state that they never occur in these cells. The same holds for the cells of the *sheath of Schwann.*

This comparison of the distribution of extracellular and intracellular leprosy bacilli permits some rather evident conclusions. It indicates that bacilli, which are for some reason outside of cells, do not ordinarily remain long in this situation or multiply rapidly in it, but promptly become intracellular, because otherwise we would expect them to be much more numerous. Their occurrence in some kinds of cells and their absence, or very occasional presence in others, is not simply a question of entry into cells with which they happen to come in contact, for lymphocytes and plasma cells, devoid of bacilli, occur side by side with monocytes which possess them. Nor is the evidence sufficient to agree with White [9] that the association between bacilli and monocytes depends on the possession in common of some chemical factor "through which they contend for the same food supply." To single out thus the monocytes and ignore the other cells, within which the bacilli are found, is unjustifiable. Moreover, reasons have been given why this explanation is probably not valid.[10, 11]

To speak of the bacilli as invading the cells which they come to inhabit is not justified because to do so implies action on their part in overcoming some sort of cellular resistance. Leprologists are agreed that the bacilli, seen in mounts of living tissue, are not motile. It is known that killed rat leprosy bacilli injected into rats become intracellular and cause lesions which resemble those brought about by living bacilli, except that they are of course limited and do not progress since there can be no multiplication

of the dead bacilli (Lowe [12]). It is therefore improbable that living human leprosy bacilli are incorporated in the cells any more actively than dead bacilli by virtue of their vitality.

As for the cells, their part may not be exactly what we would call "active." The epidermal cells are sessile and do not move about and seek out the bacilli. Endothelial cells are naturally more in the path of the organisms. Whether motile leukocytes actually make their way to them remains to be determined. The bacilli sink into the cytoplasm of some kinds of cells and not into others, and this is conditioned by the properties of the bacterial and cellular membranes.

The number of bacilli in any particular cell is not necessarily a measure of the number taken in. It is a function, not only of the intake but also of the rate of destruction of the bacilli by the cells, on the one hand, and of multiplication of bacilli in them on the other. Neither should the factor of time be ignored. The same rate of change over a long period will produce a greater result than over a shorter one. The life spans of the different sorts of bacilli-laden cells have not been determined, but they are evidently not all equal. For instance, the monocytes of the lesions exhibit few signs of death and they probably live longer than neutrophils. Considerations such as these illustrate how complex is the problem of accumulation of bacilli in globus formation.

2. *Small Globi*

The intracellular position of small globi is not in question. It is the giant globi that are so very difficult to interpret. Because the giant globi have to be considered in possible relation to the smaller ones, it is desirable to describe briefly the smaller globi first. Two types are easily recognizable.

The *cigar packs* are the smallest as well as the most numerous. They are made up of bacilli packed side by side like cigars (or better cigarettes) for their ends do not taper. As indicated in Figure 14, they constitute masses which are not globular any more than a package of cigarettes is globular. The bacilli in the packs are often of the same length so that the lateral limits of the pack, as well as the ends, seen in profile, are straight lines. Quite frequently the pack is tilted with the result that the end meets the sides at obtuse and acute angles. When many packs are ob-

served in a section through a large macrophage, for example, their long axes may lie in many directions. This is shown in the upper right hand corner of Figure 14. Common measurements of cigar packs in sections grade from 3 by 1.2 μ to 3 by 3.6 μ. Globi of this sort can be found in all of the types of cells that have been listed as containing intracellular bacilli, but they are only conspicuous components of the monocyte-histiocyte-macrophage group of cells and their derivatives.

Globi of the second type may be less numerous than the cigar pack globi. They are larger, roughly from 3.7 by 8.4 μ to 6 by 14 μ. In shape they are elongated bodies, somewhat circular in cross section with tapering ends. They somewhat resemble oat seeds and may be called *seed globi.* Because they are not found in so many types of cells as the cigar packs it follows that they are not inevitable developments of the cigar packs any more than all intracellular bacilli accumulate in cigar packs. Most noteworthy among the cells, which may contain small cigar packs but do not exhibit seed globi, are the neutrophilic leukocytes. Seed globi are uncommon in epithelial cells.

Yet no sharp dividing line can be drawn between cigar packs and seed globi. By careful selection globi can be found which appear to represent an unbroken series of transitions from cigar packs to seed globi. With the close association of more bacilli side by side the length of the globus increases because their ends are no longer held in a single plane. On the contrary, some of the bacilli extend beyond the confines of the original cigar pack and increase its length. As a seed globus takes form the packing of the bacilli becomes less close. Fluid accumulates. The clear halo often noted about the cigar packs becomes wider. Stainable material, wrongly called "Schleim" by the Germans because it is not mucus, increases in association with the bacilli. The centers of the larger seed globi appear to be less rich in bacilli than their peripheral parts. Seed globi are well illustrated in Unna's [13] unsurpassed colored plates (Figs. 260 and 261).

3. Giant Globi

Unna did not inform us when globi become large enough to be called giants, but it seems to me that those whose diameters, measured in sections, range from 15 to 150 or more μ may be so

dignified. They were discovered before the smaller ones and it is from their typical globular form that the cigar packs and seeds take the name "globi," though they themselves are not spherical. Thus Unna [13] and Marchoux [14] rightly include cigar packs under the heading of globi.

Nevertheless, from the following quotation it appears that Manson-Bahr [15] applies the term "globi" to structures different from the cigar packs: "In addition to the bacilli-bearing cells, and increasing in number with the age of the lesion, a number of brown granular bodies, larger and smaller, named 'globi,' are to be seen; these are thought to be cells in which the bacilli have perished and become granular." Clearly the cigar packs are not brown granular bodies, nor are they cells in which the bacilli have necessarily perished and become granular. Gay and Steinbach,[16] also in describing the bacilli, write that "they occur most characteristically in 'cigar bundle' masses and in colonial clumps that contain enormous numbers of rods. Most characteristic of all is the presence of both intracellular and extracellular gloeal masses, known as 'globi,' which are clumps of bacteria encased in a capsular material." Since the cigar pack globi are not extracellular gloeal masses of bacteria enclosed in a capsular material, they are apparently not globi in the meaning of Gay and Steinbach.

In tracing the origin of the smaller giant globi, which can be clearly seen to be intracellular, there is no difficulty. By selecting for examination the less dense parts of a nodule the giant cells can be studied more or less individually and their limits definitely established and, within them, the generally rounded giant globi are produced, at least in some cases, by a ballooning of seed globi. The nuclei of the giant cell are displaced usually toward one side, as the nucleus of a fat cell is displaced by enlargement of the contained fat droplet. In both, the growth in size is conditioned much more by the increase in stored material than by increase in volume of cytoplasm in the strict sense of the term. Sharp lines of demarcation are recognizable both between the rounded droplet of fluid containing the organisms and the droplet of fat, and the cytoplasms in the cells that possess them. If the included materials should escape and the cells survive, they would in all likelihood shrink to comparatively insignificant dimensions. Indeed, we do not find the giant globi developing in giant cells possessed

of much cytoplasm and many nuclei of fairly uniform size peripherally placed. Such cells, often spoken of as belonging to the Langhans type, are common in the dermal lesions of tuberculoid leprosy where leprosy bacilli are extremely rare. In cutaneous nodular (lepromatous) leprosy, with which we are dealing, they are infrequent and comparatively small. Those that do occur may contain a few bacilli and globi of moderate size, as well as occasional stellate bodies; but it would appear that they are not produced by the dilatation incident upon the formation in them of giant globi in the same manner as the giant cells under consideration, which are, by contrast, the more difficult to identify the larger the size of the contained globus. Figures 17 and 18 illustrate giant globi limited by membranes, the giant cell derivation of which can only be demonstrated in serial sections.

Though it is reasonable to assume, as a working hypothesis, that still larger giant globi are merely enlargements of the smaller ones located in even more swollen giant cells of the same nature, evidence is needed. It is with these really enormous accumulations of bacilli that Denney was chiefly concerned. He regarded them as masses of bacilli enclosed in a membrane of undetermined nature. Principal reliance was placed by him in observations made on mounts of fresh tissues. I have also examined these bodies, obtained from biopsies and mounted in physiological saline plus numerous supravital stains, without much success. When a preparation is made, sufficiently thin to study at high magnification, the giant globi are compressed and their topographical relations with other tissue components are lost. Consequently my study has been based chiefly on sections.

If the membranes that surround the largest globi are not the remains of the investing giant cells, what are they? As indicated at the beginning of this paper, Unna and his followers would have us believe that the membranes are the walls of lymphatic vessels and that the giant globi are masses of bacilli which have formed extracellularly in their lumens. The trouble is that we have but few data on the participation of lymphatics in the pathogenesis of the lesions. Leprologists seldom have anything definite to say about them, while the emphasis placed on them by leading pathologists is uneven. MacCallum [17] states that the lesions are ". . . rich in blood vessels and especially in wide lymphatics," and

Mallory [18] completes his account of leprosy without any mention of lymphatic vessels. Apparently no investigators have demonstrated the lymphatics in leprous nodules by the improved injection method devised by Hudack and McMaster.[19] Reasons for concluding that giant globi are contained in lymphatic vessels are not convincing.

One often finds (see in Figure 1) that the giant globi are disposed in a layer at a fairly definite and uniform distance from the epidermis. This layer corresponds roughly in position to that of the superficial lymphatic plexus of normal skin as recently studied by Gray.[20] In other cases, as Gray pointed out, the vessels of normal skin extend into, or better begin in, the dermal papillae, and I find that giant globi may likewise occur in this situation (Fig. 2). In still other cases there are no giant globi near the epidermis (Fig. 3). But correspondence in position of giant globi with the lymphatics of normal skin, or lack of it, means very little either way.

Globus material (bacteria and Schleim) has been frequently reported in varicose spaces which branch and have been taken to be lymphatics. Without seeing the particular specimens an opinion is hardly justified. Some appearances were encountered which may be of the same sort. Figure 6 shows, at high magnification, a large mass of globus material extending from the lower left hand corner of the microphotograph upward and to the right. It is contained within an ill-defined and branching space but the walls of the space are not smooth and endothelium-lined, as would be the case if they were those of a lymphatic vessel. Close examination shows that the globus material, partly invested in fluid, was in this instance free in the tissue and was not limited by any microscopically visible membrane. Further, it was found that this state of affairs obtained near the edge of the section where there were signs of mechanical trauma. The suggestion is advanced that the membranes, enclosing several giant globi, were ruptured and that the contents escaped into the tissue fluid and flowed together.

Examples have been noted of globi resident in spaces, the walls of which project into the lumen in a manner remotely suggestive of valves in a lymphatic vessel. Even though it must be admitted that the structures, if they are valves, may differ materially from normal valves in such a lesion, the differences nevertheless are

too great to entertain the theory that we are dealing even with altered values. The projections are neither coated with nucleated endothelial cells nor placed at an acute angle with the wall, like valves. Instead they extend directly into the lumen and appear to be the walls of giant globi which have come together and partly disappeared so that they no longer entirely bridge the gap.

Some evidence can be brought forward that the giant globi observed are not extracellular formations existing within the lumens of lymphatic vessels. They do not exhibit within their investing membranes either the scattered lymphocytes and other cells or the typical coagulum of lymph seen in sections. If, however, they are in the lumens of lymphatic vessels the normal contents of the said vessels must have been in some way banished owing to unusual conditions operative in the lesions.

It has not been possible to establish continuity between the walls of giant globi and the walls of lymphatic vessels. In attempting to do so I first followed rather isolated giant globi, like those represented in Figure 1, from section to section without finding evidence of continuity. Careful study of very thick sections, lightly stained, was not helpful. Numerous reconstructions were then made from serial sections 6 μ thick. In very rare cases one or two delicate thin walled tubes led off from the lumen containing a giant globus. This condition of affairs is illustrated at high magnification in Figure 19. A giant globus is shown in what looks like a clear space, limited by an ill-defined wall, which is incomplete below where the tube leaves and passes to the left. The walls of this tube are smooth and quite definite in the original preparation, but they could not be traced more than about 12 μ. Whether such a tube is a lymphatic capillary or not cannot be stated positively. But, almost always, the walls of the globi are complete, without any outpouching, though it is not unusual for the spaces containing giant globi to unite.

To make reconstructions of giant globi in deeper lesions containing large numbers of them is extraordinarily difficult by reason of the absence of suitable landmarks. However the giant globus in the center of Figure 5 was observed to be in a lumen which communicated peripherally with seven other lumens, each containing a somewhat smaller giant globus. Two openings are represented on the right above and below. Unna observed these

formations and designated them *cactus forms* of giant globi. The peripheral giant globi are attached to the central globi like the short thick leaves of a cactus to its branches.

Application of Mallory's aniline blue collagen stain and of Masson's modification of Mallory's stain demonstrates that the walls of these giant globi are not strengthened by collagenic fibers in a manner reminiscent of lymphatic vessels. Weigert's elastic tissue stain brought to light a few elastic tissue fibers but they were not definitely related to the walls of the globi. The absence of a characteristic fibrous backing for the walls and of any vestiges of muscle is evidence that the walls are not those of lymphatic vessels, but does not exclude an interpretation that they may be those of distended lymphatic capillaries. This is, however, improbable because silver impregnations failed to reveal, in the walls, lines of cement substance even remotely comparable to those which outline the component endothelial cells of lymphatic capillaries.

Passing now from cutaneous lesions to those in regional lymph nodes, conditions are found which Unna may have taken into consideration. Here, the globi may be even larger than in the dermis. Compare Figure 4 with Figure 1 taken at the same magnification. In the former a larger and a smaller row of giant globi are shown within penetrating lymphoid sinuses. Examination of the sections at high magnification has, however, shown so many examples of the development of these globi within the cytoplasm of giant cells that an intracellular is much more plausible than an extracellular origin.

Figure 10 represents a section through a giant cell formed from the littoral cells of a peripheral lymphoid sinus. The body of the lymph node is to the right. The lumen of the sinus extends from the upper right margin of the microphotograph downward and to the left. It is much dilated by a giant cell in the cytoplasm of which there are two globi. These may be recognized by the quarter moon shaped masses of bacilli and Schleim, the concavities of which face to the right. The lumen can be traced around the right hand side to the lower border of the giant cell. In this section a thin band of tissue intervenes so that the lumen appears interrupted before it extends to the numeral "10," but in neighboring sections there is continuity of the lumen.

Figure 11 is from a section colored by Masson's stain. Extending down the middle and into the substance of the node is a band of collagenic fibers. On either side of the band is a penetrating lymphoid sinus. Applied to the right wall of the sinus on the right is a large giant cell. Within the cytoplasm of this cell is a giant globus, somewhat wider above than below. The wall of the giant cell enclosing the globus is actually complete on all sides but unfortunately the cell wall between the globus and the lumen of the sinus is not shown in the microphotograph.

It is not uncommon to find scattered masses of globus material within the lumens of lymphoid sinuses, as on the left of the collagenic band in Figure 11, but it appears that this is due to the rupture of giant cells containing globi rather than to their formation extracellularly within the lumens.

Only short stretches of afferent and efferent lymphatic vessels outside of the capsules of lymph nodes were examined. Although no giant globi were discovered in them, I am not prepared to say that they would not have been observed had the search been more extended. However, as previously noted, lymphatic vessels observed in association with cutaneous nodules were not found to contain globi and the endothelium of lymphatic vessels is different from, and much less phagocytic than, the littoral cells of the lymphoid sinuses which do form giant cells and intracellular globi. The littoral cells are components of the reticuloendothelial system, whereas the endothelial cells of lymphatic vessels are not.

A survey of the available autopsy specimens shows that globi which are well over 15 μ in diameter, yet not as large as those in the skin and lymph nodes, occur in parts of tissues devoid of lymphatic vessels. They are seen for example in the white and red pulp of the spleen, while lymphatics are restricted to the trabeculae, and in the cortical substance of lymph nodes distant from lymphoid sinuses (Figs. 7 and 8). A few may occur near the centers of hepatic lobules, though the lymphatics are limited to their periphery. Perhaps a more detailed search might reveal giant globi in other alymphatic areas.

Summing up, no evidence has been found that giant globi are formed extracellularly within the lumens of lymphatic vessels. On the contrary a number of observations have been reported which point to the conclusion that they develop intracellularly

within giant cells. In many cases they are greatly enlarged seed globi but it is possible that some of them are formed without preliminary orientation of the bacilli either as seed globi or cigar packs. Certainly many cigar packs and seed globi do not transform into giant globi because if they all did giant globi would be ever so much more numerous than they actually are.

The giant cells in the dermal nodules are of the type that have long been regarded [21] as fusions of mononuclear cells (monocytes). In the lymphoid sinuses of lymph nodes they are produced from the littoral cells, while within the cortical substance of lymph nodes they may result from the fusion of cells other than monocytes, perhaps of reticular cells. It is interesting to encounter in the latter "spiculated" or "stellate" bodies (Fig. 9) which resemble those in the dermal giant cells and occur in other conditions as well as in leprosy.[18, 22] The autopsy material available does not permit statements as to the genesis of giant cells possessed of globi in still other parts of the body. Muir and Chatterji [23] do not make a good case for their assertion that "there is reason to believe that the presence of a giant cell in any leprous lesion shows that the part affected is of nerve origin." Neither am I able to agree with them that it is, ". . . not at all unlikely that the apparent arrangement of the nuclei of giant cells in leprosy is due to the endothelial cells of obliterated capillaries. . . ." It is clear, however, that the cells of the reticuloendothelial system are most involved and that the tendency to fuse is manifested even after giant globi have developed within the said giant cells. This is illustrated in Figure 7 as well as in Figure 5 of a cactus form of giant globus.

4. Significance of Globi

It has been said by Manson-Bahr that the number of "brown granular bodies" (which are giant globi) increases with the age of the lesion, but to supply proof is difficult. While statements are available as to the duration of the disease for all cases from which material was collected, the ages of the individual lesions are not known. In Table II is shown the result of an effort to discover whether there is any relation between the duration of cutaneous nodular leprosy and the incidence of giant globi.

The relative numbers of giant globi are indicated by the number

of + signs, but the globi were not systematically counted per unit area of cytoplasm. Estimates were made of their frequency based on the study of many sections. If a larger number of blocks of tissue had been examined a negative report might have been converted into a positive one. Especially in the older individuals, the lesions may have been present for a considerable time before a positive diagnosis was reached. But, speaking generally and admitting the possibility of exceptions, the figures are consistent

TABLE II
Incidence of Giant Globi

Case number	Age	Duration	Giant globi
	yrs.	*yrs.*	
15	12	4	−
3	42	4	+
5	36	5	++
13	44	7	−
7	30	8	+
14	17	9	−
16	22	9	−
19	23	9	+
4	30	10	++
20	35	16	+++
17	25	18	+
6	35	19	++++

with the idea that there is an increase in the incidence of giant globi with increase in the duration of the disease.

Obviously the time required for extracellular bacilli to enter the cells, form cigar pack globi, seed globi and giant globi remains to be determined and the ultimate fate of the giant globi requires investigation. The processes may be more rapid in more susceptible persons. It would not be surprising if they are accelerated by fever in the "lepra reaction."

IV. Discussion of the Intracellular Behavior of Acid-Fast Bacilli

Since globus formation in human leprosy is essentially an unusual accumulation and arrangement of bacilli within cells, it should be interpreted in perspective, having in mind the intracellular behavior of still other acid-fast bacilli.

It is instructive to contrast rat leprosy and Johne's disease of

TABLE III

Comparison of Acid-Fast Bacterial Diseases

	Human leprosy	Water buffalo leprosy	Rat leprosy	Johne's disease of cattle
Incubation period	5–10 yrs. or longer [24]	Onset not observed	4–6 months	Never less than 1 yr., often over 2 [32]
Age incidence	Chiefly young adults	21 cases apparently all adults [7]	Mostly adults	Chiefly young adults [32]
Sex incidence	In adults reported higher in males, but in children about equal [35]	Data lacking	In question [29]	Data lacking
Course	Several types; in one (tuberculoid) bacilli are infrequent, in another (lepromatous) they are very numerous	One type many bacilli	One type many bacilli	One type many bacilli much emaciation
	Usually very chronic afebrile	Similar	Similar	"As a general rule, the disease is afebrile" [32]
	Febrile type recognized	Febrile type reported but temporary	Not reported	
Lesions	Granulomatous. May be widespread including often nasal mucous membrane; intestine usually not involved [36]	Granulomatous. Skin and occasionally in nasal mucous membrane	Granulomatous, skin, and/or viscera. Occasionally nasal mucous membrane in natural disease, gastrointestinal tract apparently unaffected [29]	Granulomatous. Intestinal mucous membrane
	Nerve involvement may be marked	Not reported	Not reported	Not reported
	Regional lymph nodes	Regional lymph nodes	Regional lymph nodes	Regional lymph nodes
	Fibrosis	Similar	Similar	Fibrosis absent or slight
	Necrosis in large nodules	Similar	Similar	No evidence of necrosis
	Calcification occasional	"Always accompanies necrosis" [7]	Infrequent	Not mentioned

	Calcification occasional	"Always accompanies necrosis" [7]	Infrequent	Not mentioned

	Acid-fast rods and granules	Acid-fast rods and dark red granules + angular forms	Acid-fast rods and granules	Smaller acid-fast rods and granules
	Cultivation?	Attempts –	Cultivation?	Cultivation +
	Chiefly intracellular	Similar	Similar	Similar
	In greatest variety of cells including vascular endothelium	Less than human leprosy in variety of cells not in vascular endothelium	Less than human leprosy in variety of cells. Vascular endothelium "was never found infected" [30]	In smallest variety of cells
	Form cigar pack, seed and giant globi containing Schleim	"Bundles," and globi even larger than in human leprosy embedded in fat	Rosettes without Schleim or fatty material	Peripheral accumulations without Schleim or fatty material
Bacilli	Fluid accumulation slight about cigar packs and in and about seeds, but marked within investing membrane of giant globi	Even more pronounced within membrane of giant globi	Slight within membrane limiting large rosettes	Not noticeable
	Loss of acid-fastness sometimes seen in bacilli wherever located but particularly in giant globi	No data	No non-acid fast forms found	Not mentioned by Hagan [32]
	Granulation very common, not all degenerative	Said by Lobel to be degenerative	As in human leprosy	Inconspicuous perhaps owing to small size of organisms
Transmissible to	Hamsters [27, 28]		Mice of mixed breeds [31]	Sheep in which species it may occur naturally

cattle with human and water buffalo leprosy (Table III). All four have long periods of incubation (or latency), are local infections, usually without fever, which become chronic and spread slowly, and are caused by acid-fast bacilli which infest mainly cells of the reticuloendothelial system; but in a study of bacteria-cellular relationships the differences are as significant as the similarities. Thus, the human disease is most diversified both in clinical types and in the variety of cells involved, while Johne's disease is most definitely limited to the monocytes and histiocytes.

How far globus formation is conditioned by the bacilli on the one hand, and the cells which they infest on the other, is a question that will for a long time remain unanswered. A few of the many factors which may operate as between the human leprosy bacilli and several varieties of cells in the same host have been mentioned, but the differential behavior of a single sort of bacillus in cells of the same kinds but in different species is even more important. Unhappily there are but few data because when injected into species in which they are not native the bacilli apparently find it very difficult to take hold. But active and sustained multiplication does follow the inoculation of human leprosy bacilli into hamsters (Adler,[27] Burnet [28]) and of rat leprosy bacilli into mice of mixed breeds (Sellards and Pinkerton [31]). In both instances the bacilli became lodged within the same monocyte-histiocyte-macrophage group of cells that they originally inhabited in their natural hosts.

Evidence is scanty on the ability of human leprosy bacilli to form globi in hamsters, as is their custom in humans. Adler made no reference to globus formation, while Burnet, briefly and in passing, reported the presence of globi in smears of subcutaneous tissue which he illustrated by a figure in which nothing resembling a globus can be seen. Both were mainly concerned with transmission.

It has been found that in rat leprosy the bacilli are sometimes arranged in very characteristic rosettes.[33] These are dense intracytoplasmic masses of bacilli disposed so that they stretch outward, or radiate from a central focus as is represented in Figure 16. Sellards and Pinkerton, likewise primarily interested in transmission, have reported neither the presence nor the absence

of rosettes of rat leprosy bacilli in mice. Until, therefore, a purposeful search is made for globi of human leprosy bacilli in hamsters and for rosettes of rat leprosy bacilli in mice — both of course after inoculation with the respective bacilli — it cannot be said whether these formations are conditioned by the bacilli or by species differences in cells belonging to the same category.

Very intriguing is the similarity, described by Lobel, between the globi in water buffalo leprosy and those in human leprosy. He reports the grouping of bacilli "in typical bundles or globi" and adds that the bundle-like arrangement does not suggest "cigar-bundles." Just how close his bundles are to cigar pack globi in human leprosy neither his text nor his illustrations makes clear. The "bacillary globi are," he states, "embedded in fat." This is not the case in human leprosy in which the bacilli are associated with "Schleim" which is not fatty. The globi in water buffalo leprosy occur within multinucleated giant cells, as in human leprosy, while the "great globi" (much larger than in human leprosy) are found "in large cavities," the nature of which he does not specify. The water buffalo material at my disposal is poorly preserved and limited to a few blocks of tissue. I found no evidence of the extracellular development of globi within lymphatic vessels or elsewhere. In one instance a giant globus occurred within a thick layer of stratified epithelium (Fig. 13) in which there are ordinarily no lymphatics. It is to be hoped that Lobel will soon supply further details. At present the resemblance in the bacilli, in the globi they form and in the whole disease reaction, raises the question whether water buffalo leprosy is a disease entity or an infection of water buffalos by human leprosy bacilli.

In Johne's disease the bacilli frequently give rise to discrete rounded colonies in the cytoplasm of the infested cells. Because these resemble more in shape small clumps of heartwater Rickettsiae in endothelial cells [34] (and intracellular masses of small organisms occur in a variety of diseases) than they do cigar packs, seed or giant globi in leprosy, it would confuse the issue to call them "globi." In addition I have observed a more unusual accumulation of bacilli in the lesions of Johne's disease, which appears to be at least partly conditioned by the cells in which they live. These monocytes, macrophages or histiocytes, whichever we like

to call them, quite frequently show central clear areas about which the bacilli are heaped up in the peripheral cytoplasm. For brevity and convenience the peripheral ring of bacilli may be styled a "peripheral body." One of these is illustrated in a binucleated cell centrally placed in Figure 15. A nucleus can be seen below and to the right, while traces of another are visible in the microphotograph below and to the left. Bacilli are evidently densely packed together in the peripheral cytoplasm leaving a central zone free. This is the appearance in section, but considering the whole cell, the peripheral body, made up of bacilli, is the thick wall of a rather irregular sphere limiting a center containing few if any bacilli.

In the specimens studied the cells containing peripheral bodies were of fairly uniform size and quite numerous, but, since they are yielding structures in a granulomatous lesion, they are not always spherical. The peripheral arrangement of bacilli about the clear central area reminds us strongly of a somewhat similar peripheral arrangement of neutral red granules around a central area in monocytes and macrophages supravitally stained with neutral red. Why neutral red granules become oriented in this way is not known, but it seems to be conditioned by the cytoplasm in which they find themselves. In the central clear area a "central body," or centrosome or diplosome can often be located from which the neutral red granules appear to be held at a distance. Unavailing was a hunt for central bodies in cells possessed of bacilli. However the fixative had not been chosen with this in view. It should have been diluted in accordance with Fry's [35] suggestions. Even in the absence of microscopically visible central bodies it is logical to assume that the similar orientation of the bacilli to that of the neutral red granules in cells of the same sorts is occasioned by similar conditions in the cytoplasm.

For some reason the much larger bacilli of human, water buffalo and rat leprosy do not become characteristically arranged in peripheral bodies. Perhaps they are not so subject to the orienting force operating from the central body area. Occasionally in human leprosy the bacilli are accumulated in the peripheral cytoplasm of cells of the same variety and of approximately the same size as the one shown in Figure 15, but never were they observed to form so dense a mass.

In the present state of our knowledge the cigar, seed and giant globi may be regarded as specific for human leprosy, the rosettes for rat leprosy, and the peripheral bodies for Johne's disease. The bundles of bacilli and globi observed in water buffalo leprosy either indicate a definite disease of water buffalos or an extension of human leprosy to them. Thus far, no clumping of bacilli, even remotely similar, has been reported in human or animal tuberculosis. In all four conditions these peculiar bacillary masses are intracellular formations in cells of the reticuloendothelial system. There is no evidence that any of them develop extracellularly within the lumens of lymphatic vessels.

Although the significance of the formations remains obscure, it is possible that they are not essential stages in the life cycle of the respective acid-fast bacilli. In tuberculoid leprosy bacilli may be of extremely rare occurrence and globi absent. In rat leprosy rosettes are sometimes not seen. Whether they would always be found, if the examination were sufficiently thorough, is doubtful. More data are needed concerning the incidence of peripheral bodies in Johne's disease. Only 21 cases of water buffalo leprosy have been studied by Lobel.

There is no definite correlation between occurrence of the formations and loss in acid-fastness, or granulation, of the respective bacilli. In human leprosy quite large areas of lesion may show loss of acid-fastness of many bacilli both outside and within all varieties of globi. Moreover, in all situations non-acid-fast may exist side by side with acid-fast bacilli. It is unsafe to extend to human leprosy conclusions as to the significance of loss of acid-fastness in tuberculosis.[36] Rat leprosy is different in so far that there is typically no loss of acid-fastness, while in the lesions of water buffalo leprosy and Johne's disease loss of acid-fastness has not been reported.

Human and rat leprosy are much alike in the frequency of a particular type of granulation of bacilli. In it, the bacilli retain their usual length and diameter but they are made up of granules separated at regular intervals by non-acid-fast materials. This condition is commonly encountered in large stretches of tissue including both globi and rosettes. Apparently it is not an expression of death of the bacilli for it occurs in actively extending lesions. In water buffalo leprosy Lobel refers only to granular

degeneration of the bacilli. He says that this phenomenon is more marked than in human leprosy. It may be that he is dealing with a kind of granulation comparable with that which seldom or never pervades the whole lesion in human leprosy, is characterized by granules, often of larger diameter than the bacilli and staining more deeply, and which is quite possibly a sign of bacterial degeneration. He reports that the granular degeneration products as well as the rods "are grouped in typical bundles, or globi," but he does not inform us whether the granules occur in the absence of such grouping. If his interpretation be correct the typical bundles are composed at least partly of dead bacilli incapable of multiplication. In Johne's disease granulation of the bacilli is less conspicuous than in the other diseases because the organisms are much smaller. A method, recently devised [37] for collecting the bacilli of rat leprosy, free from fibrous and cellular materials, may open the way for more accurate determinations of acid-fastness and granularity.

Large acid-fast droplets occur in the visceral lesions of human leprosy. One of these is represented in black in Figure 12 because it was deeply stained in the original section. Whether they are formed through coalescence of smaller droplets resulting from the degeneration of bacilli remains to be determined. Comparable droplets were not seen in either rat leprosy or Johne's disease. They should be sought in water buffalo leprosy.

Accumulation of fluid about intracellular clumps of bacteria is a common finding in many diseases. In human leprosy this is very marked. It begins about the cigar packs and, in the giant globi, the bacilli are suspended in fluid. Enormous dilatation of the giant cells, in which they reside, is caused more by the increase in fluid than by the number of bacilli. The fluid, when fixed and stained, exhibits the amorphous or granular material, known as "Schleim," which has not been described in water buffalo leprosy and does not occur in rat leprosy or Johne's disease. This fluid accumulation in human leprosy and, according to Lobel, the heaping up of fat in water buffalo leprosy are factors in the great size of the globi. In rat leprosy there is less intracellular fluid accumulation and in Johne's disease little, if any. Since cellular permeability can be increased by Na and K ions and decreased by bivalent cations such as Mg and Ca,[38] it is possible that the fluid

accumulation can be altered experimentally. Spectrum analysis may prove useful.[39]

V. Summary

1. In dermal lesions the vast majority of bacilli are intracellular. They occur mainly in the cytoplasm of monocytes, macrophages (epithelioid cells), giant cells, "foam" or Virchow cells, vascular endothelial cells and neutrophilic leukocytes. Occasionally they are found in epithelial cells, the endothelial cells of lymphatic vessels, fat cells and the mesenchymatous cells of the perineurium, epineurium and endoneurium. It is doubtful whether they occur in blood and tissue eosinophils and basophils and in cells of the sheath of Schwann. They are not seen in lymphocytes, plasma cells and Russell body cells. In lymph nodes the bacilli occur in those of the above mentioned cells that are present plus reticular, littoral and giant cells. The survey of other tissues, obtainable only at autopsy, is incomplete.

2. The conditions that determine the entry of bacilli into cells are not known. It is not simply a question of contact, or of contending with the cells for the same food supply, nor is it likely that the bacilli actively invade the cells. The number of bacilli in any particular cell is not necessarily a measure of the number taken in. Several factors operate including the rates of destruction and multiplication of bacilli within the cells and the time during which they operate.

3. Within the cytoplasm bacilli may be accumulated without definite order, or as cigar pack, seed or giant globi. The latter may remain solitary, or may partly coalesce, giving rise to the cactus form of giant globus.

4. Cigar packs are made up of bacilli closely pressed together side by side and are generally of about the maximum length of bacilli. They are most noticeable in macrophages, reticular cells and giant cells, but a few bacilli arranged side by side are occasionally encountered in all the other cells that possess bacilli.

5. Seed globi are wider and longer than the cigar packs with ends that tend to taper. They also contain more fluid and some Schleim is present. Since they are not found in all of the cell types that contain cigar packs, it follows that they are not inevitable developments of cigar packs.

6. Giant globi are much larger and more rounded than the seed globi owing to the accumulation of more fluid in which there is also more Schleim. Some of them are enlargements of seed globi within cells possessed of several nuclei. But, as the giant globi increase in size, the cytoplasm of the multinucleated cells containing them does not increase proportionally in volume, nor is there a parallel increase in the number of their nuclei. Consequently, the cells in question, with progressive distention, look less and less like cells of this category in much the same manner that a fat cell differs from a slim mesenchymatous cell before it accumulates fat. It is for this reason that the investments of the largest giant globi are difficult to identify.

7. Against the concept that giant globi are lodged in lymphatic vessels or capillaries is a good deal of negative evidence. The investing membranes are not made up of endothelium nor are they backed by fibrous tissue as in small lymphatic vessels. It is not feasible to trace continuity between the investment of a giant globus and the wall of a true lymphatic. In the lumen with the globus no lymphocytes or coagulated lymph are seen. No giant globi are observed inside vessels that can be identified as lymphatics. But giant globi of moderate dimensions do occur in tissues ordinarily devoid of lymphatic vessels such as the pulp of the spleen and the central parts of hepatic lobules.

8. Within the substance of lymph nodes, where there are no endothelium-lined lymphatic vessels, giant globi develop within giant cells in the cortical substance distant from the lymphoid sinuses and particularly large ones from within giant cells originating from the littoral cells of the sinus walls. The former sometimes exhibit stellate bodies indistinguishable from those in giant cells of the dermis. The conclusion is advanced that giant globi develop intracellularly in giant cells derived from components of the reticuloendothelial system, monocytes, histiocytes, macrophages, reticular cells and, as representatives of special endothelium, littoral cells of lymphoid sinuses.

9. Finally, comparison of human leprosy with water buffalo leprosy, rat leprosy and Johne's disease is instructive. Like human leprosy, these diseases have long periods of incubation, the lesions are chronic granulomas and the bacilli are chiefly intracellular, many of them living in a kind of symbiosis with the cells. Globi

in human leprosy resemble, in some respects, those in water buffalo leprosy. They differ markedly from the rosettes in rat leprosy and the peripheral bodies in Johne's disease. There is no evidence in water buffalo leprosy, rat leprosy or Johne's disease that these distinctive aggregations of organisms are formed extracellularly within the lumens of lymphatics. At least the smaller globi in water buffalo leprosy, and all the rosettes and peripheral bodies in rat leprosy and in Johne's disease, develop intracellularly in cells of the reticuloendothelial system much as the globi do in human leprosy.

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DESCRIPTION OF PLATES

PLATE 36

FIG. 1. Giant globi appear as large vacuoles incompletely filled with deeply stained material. These globi form a layer the outer surface of which extends parallel with the epidermis for the length of the section (2.5 cm.).

Case 29. Formalin-Zenker fixation and Ziehl-Neelsen-hematoxylin stain. × 230.

FIG. 2. Giant globi, containing somewhat less deeply stained material, which is not arranged in the form of a definite layer. Some closely approaches the epidermis.

Case 10. Fixation 10 per cent formalin and Ziehl-Neelsen-hematoxylin stain. × 230.

FIG. 3. Section showing ironed out epidermis, Unna's marginal layer with venule and richly cellular lesion beneath. No large globi are seen like those in Figs. 1 and 2.

Case 4. Tissue fixed in 10 per cent formalin and section stained with Ziehl-Neelsen-hematoxylin. × 230.

FIG. 4. Connective tissue septum penetrating into a lymph node with three giant globi in the lymphoid sinus on the right and one on the left. Note that these are much larger than the giant globi represented in the dermis (Figs. 1 and 2).

Case 21. Tissue fixed in formalin-Zenker and section stained with Ziehl-Neelsen-hematoxylin. × 230.

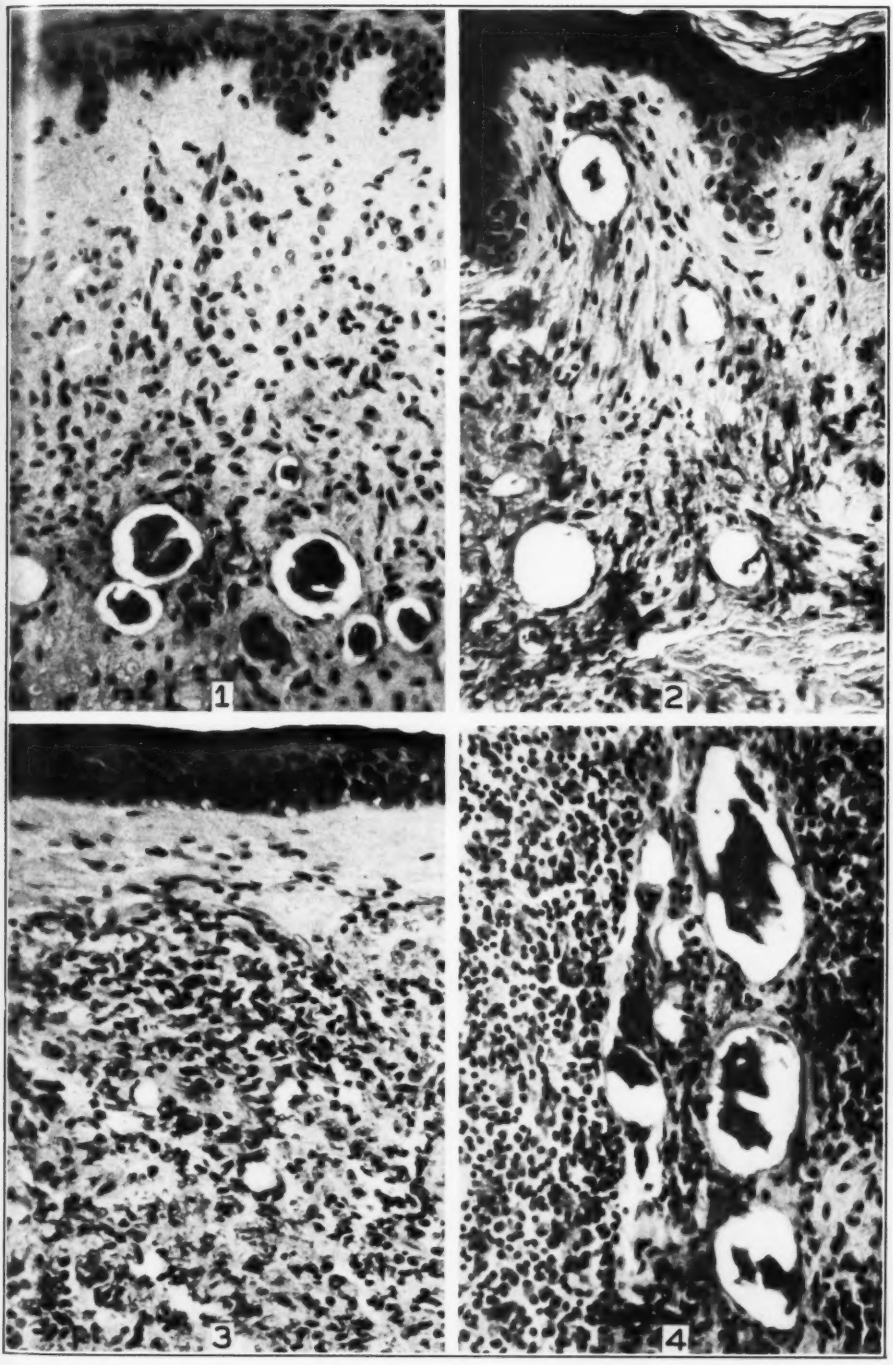
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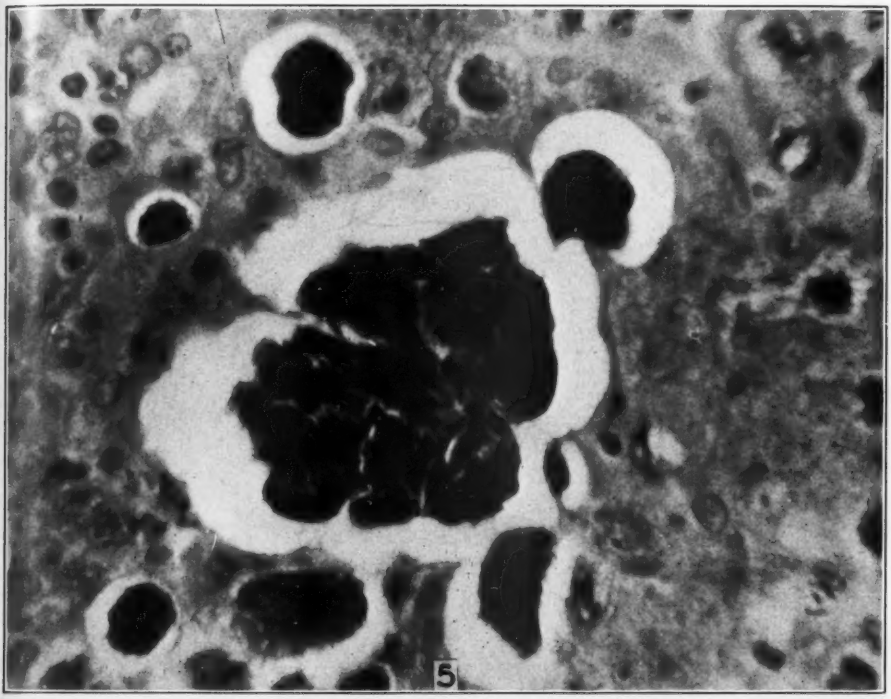
PLATE 37

FIG. 5. Cactus globus in a densely cellular lesion about 11 mm. beneath the epidermis.

Case 6. Fixed in Regaud's fluid and stained by Carpano's method. × 800.

FIG. 6. Globus material escaped from retaining walls and free in tissue fluid. It is not within a lymphatic vessel because it is not limited by a cellular membrane.

Case 7. Tissue fixed in 10 per cent formalin in absolute alcohol and section stained with hematoxylin and eosin. × 1400.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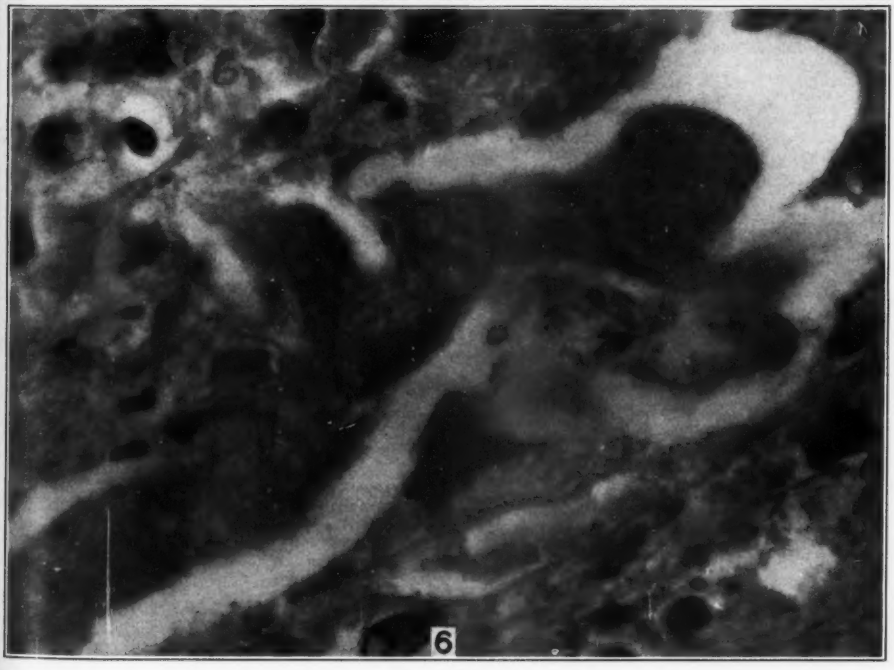
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PLATE 38

FIG. 7. Giant globi in cortical substance of a lymph node. The smaller ones to the left are partly confluent and form a cactus globus. The larger one to the right, of which only part is shown, is included within a giant cell, several nuclei of which are seen above.

Case 21. Tissue fixed in formalin-Zenker and section stained with Ziehl-Neelsen-hematoxylin. × 800.

FIG. 8. Giant globus from cortical substance of same lymph node in a section similarly stained. The nuclei of the giant cell containing the globus are clearly shown below and to the right. × 800.

FIG. 9. Giant cell from cortical substance of same lymph node in a section colored by Masson's stain. In the original preparation the stellate body in the lower left part of the giant cell exhibited a bright red stained center with blue radiating strands. Just above the stellate body is a clear area with faintly stained center. This is a beginning giant globus. To the right of it are several nuclei of the giant cell. × 800.

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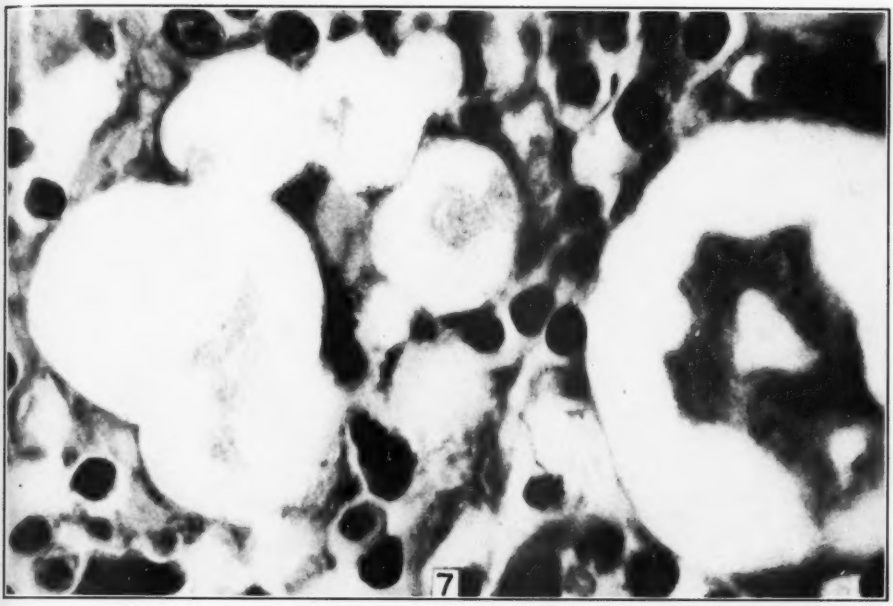
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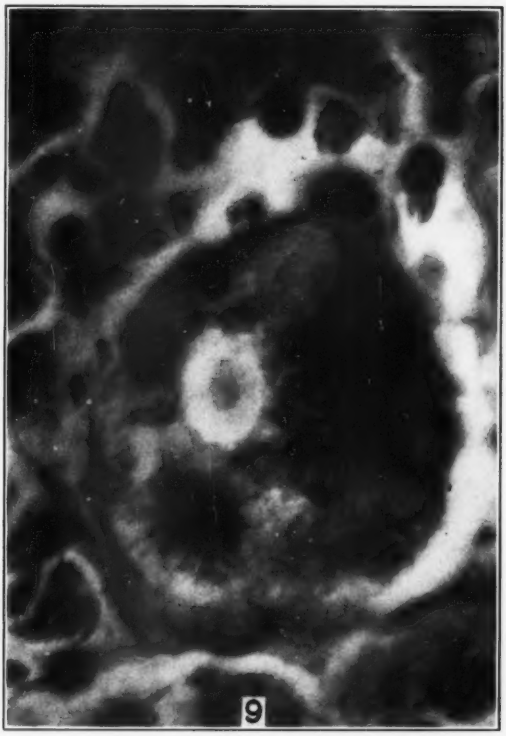
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PLATE 39

FIG. 10. Giant multinucleated cell in a peripheral lymphoid sinus of a lymph node. The cell contains two giant globi with masses of material shaped like crescents in the left hand parts of each. The nuclei of the giant cell are below and to the left of the two giant globi, and this portion of the giant cell is clearly pressed against the wall of the sinus. The upper, right, and part of the right lower margins of the giant cell face the lumen of the sinus. This lumen extends upward and leaves the upper right margin of the figure. In the next section it is continuous with the lumen of the sinus which leaves the lower left margin of the figure. From the same specimen as Fig. 7. × 800.

FIG. 11. Another connective tissue septum penetrating into the substance of a lymph node, comparable with that shown at low magnification in Fig. 4, with lymphoid sinuses containing a giant globus on either side. The globus on the right has been formed by the confluence of two within a giant cell, the right hand margin of which is close to the wall of the sinus. From the same lymph node as Fig. 4, but the section was stained by Masson's method. In the original the dense band of collagenic fibers in the septum was colored blue. × 800.

FIG. 12. A giant globus in the substance of a lymph node distant from both peripheral and penetrating sinuses is represented above and the margin of another on the left. No traces of giant cells are shown. Below is a large spherical lipoid droplet. Same tissue and stain as Fig. 11. × 800.

FIG. 13. A giant globus within stratified epithelium from a case of water buffalo leprosy. This is presented because there is no evidence that the globus is within a giant cell and stratified epithelium is ordinarily both avascular and alymphatic.

Tissue fixed in 10 per cent formalin and section stained with Ziehl-Neelsen-hematoxylin. × 800.

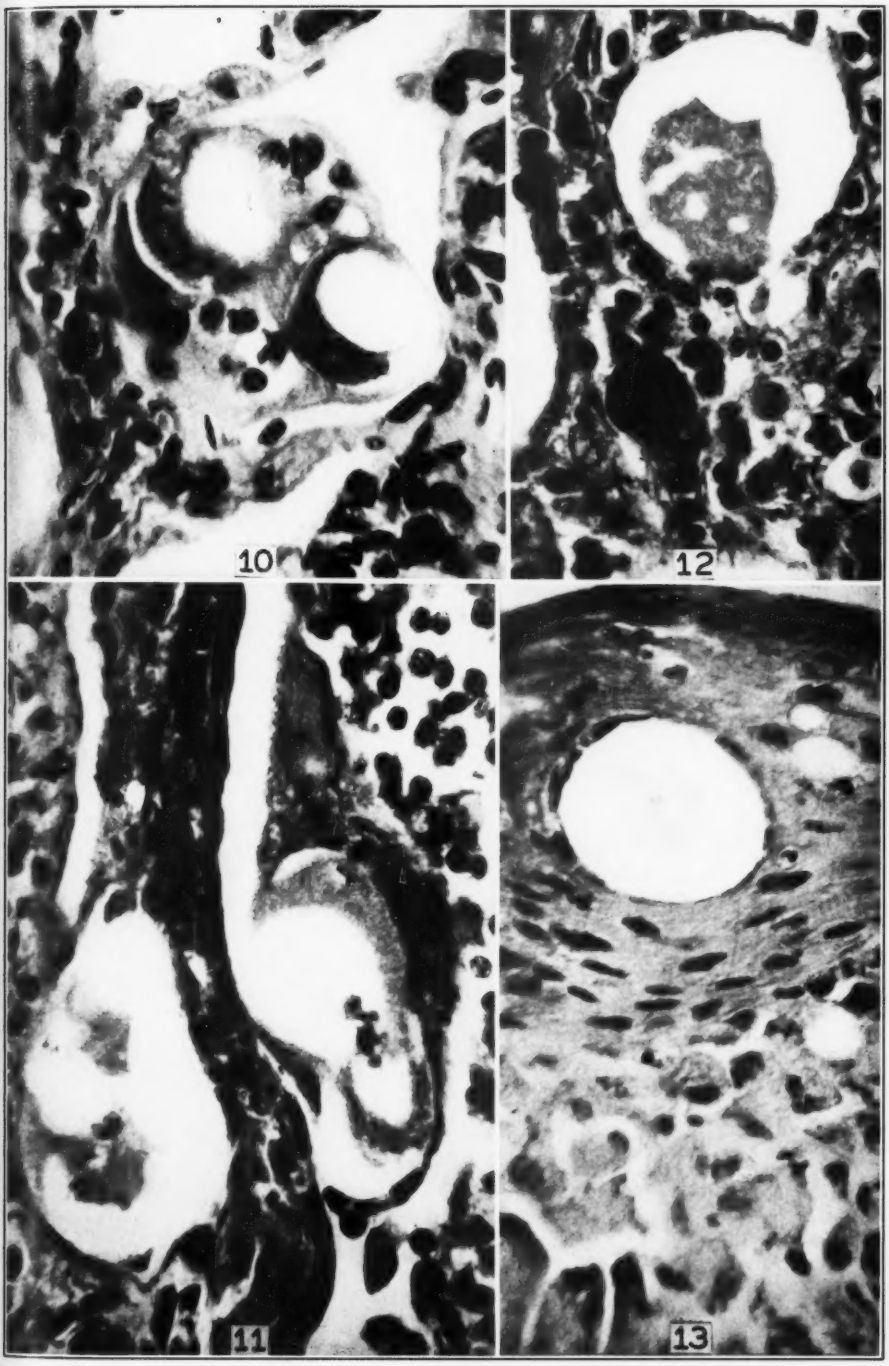
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PLATE 40

FIG. 14. Cigar packs of bacilli within monocytes and macrophages in the center of a large nodule.

Case 17. Tissue fixed in Zenker's fluid less acetic acid and section stained with Ziehl-Neelsen-hematoxylin. × 1400.

FIG. 15. Johne's disease. In the center is a binucleated cell with nuclei below to the left and to the right. They appear lighter than the peripheral cytoplasm charged with bacilli. In the center is a clear area about which the bacilli are arranged in a kind of wide halo.

Section stained with Ziehl-Neelsen-hematoxylin. From material sent by Dr. Hagan. × 1400.

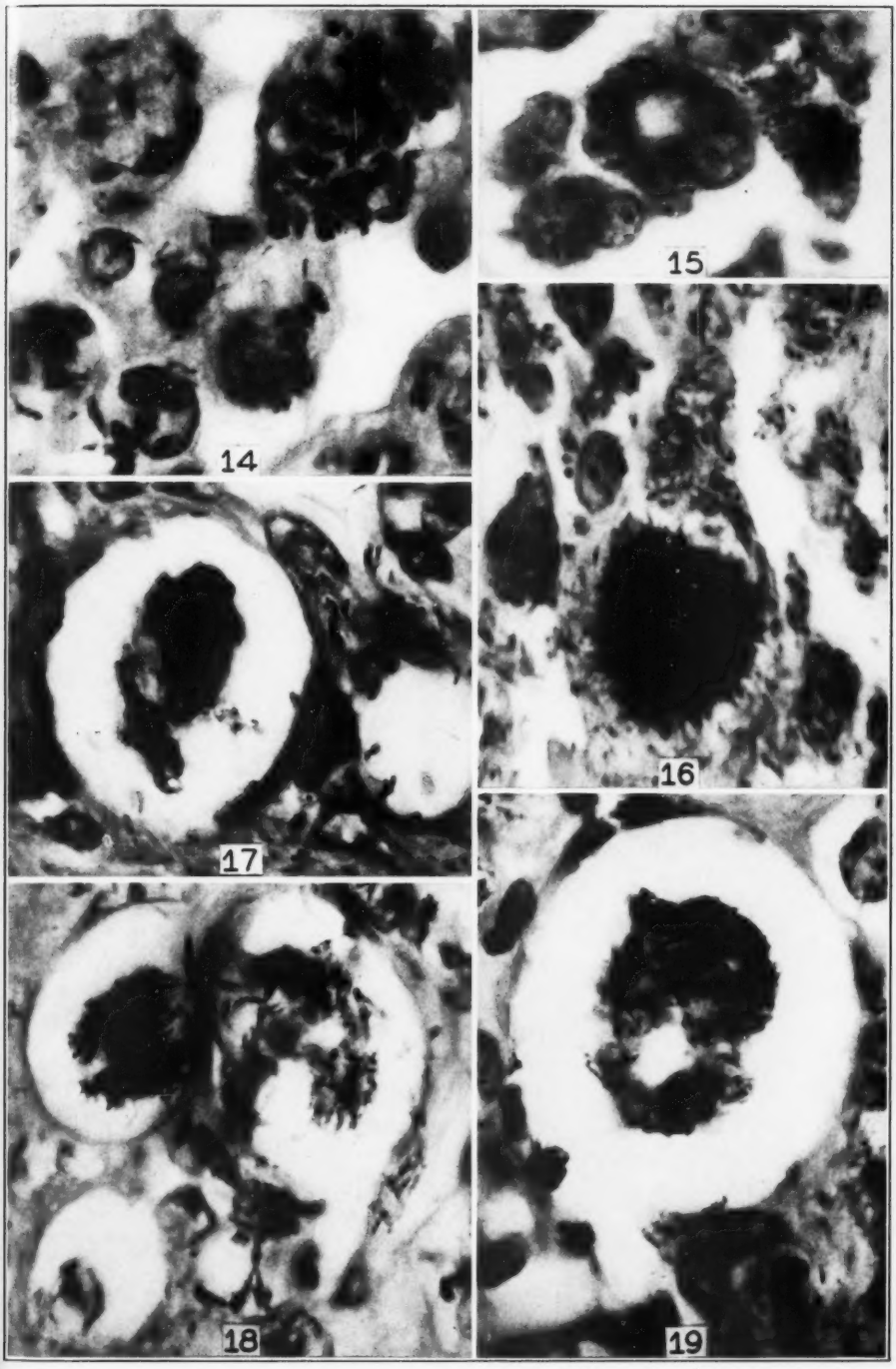
FIG. 16. Rat leprosy. Rosette made up of densely packed radiating bacilli within a multinucleated cell. Comparison with Figs. 14 and 15 shows how different is the arrangement of acid-fast bacilli of different sorts within cells of the same type. Subcutaneous nodule removed from a rat 6 months after injection of emulsion of bacilli.

Tissue fixed in 10 per cent formalin and section stained with Ziehl-Neelsen-hematoxylin. × 1400.

FIG. 17. Two giant globi in a subcutaneous nodule. There are several nuclei in their walls. One, to the lower right of the larger globus, is much elongated, flattened and deeply stained. From the same specimen as Fig. 3 but farther from the epidermis. × 1400.

FIG. 18. Three giant globi from the same specimen as shown in Fig. 17. No nuclei are to be seen in their walls. × 1400.

FIG. 19. A larger giant globus, also from the same specimen. The bacilli are clumped in the center leaving a peripheral zone free. The wall of the globus is continuous to the left, below and to the right, but above these is an opening into a narrow, bent, thin walled tube which can be followed only about 15 μ. × 1400.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PATHOGENESIS OF HERPES SIMPLEX VIRUS INFECTION IN CHICK EMBRYOS *

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The present observations on the pathogenesis of herpes virus infection are presented because they are based on new methods of approach and because they throw additional light both on the potentialities of the virus itself and on the mechanisms of virus infection in general. Previous observations on the behavior of herpes simplex virus under experimental conditions have been of importance in the understanding of the pathogenesis of herpetic and certain other virus infections of the nervous system. For example, evidence of an axonal transmission of herpes virus from a peripheral focus to the brain in rabbits has given a reasonable basis for explaining the phenomena of herpetic encephalomyelitis and constitutes a foundation for present views of the pathogenesis of poliomyelitis, rabies and several other neurotropic virus diseases.[1] The idea of axonal transmission is based on the hypothesis of cytotropism of viruses, that is, the requirement of the internal environment of living susceptible cells for virus multiplication, which is well substantiated.

Experiments with herpes virus heretofore have been concerned for the most part primarily with neuronal transmission and little or no evidence exists for a generalization of the infection by way of the blood stream or by other means. Dawson,[2] Saddington,[3] and others [4, 5] have reported the susceptibility of the chorioallantois of chick embryos to herpetic infection. Dawson maintained the virus on membranes in series and described the gross and microscopic membranal lesions. Burnet, Lush and Jackson [4] reported an increase in the virulence of this virus for embryos, a slight diminution of virulence for mice, but no change in virulence of the HF strain for rabbits during membranal transfers. Burnet and Lush [6] and more recently Schaffer and Enders [5] used embryonic membranes of chicks to titrate antiherpetic serums.

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In all of these studies observations were limited to the membranal lesions and no effort was made to determine infection of the tissues and organs of the embryo proper. My report is concerned especially with an analysis of the mechanisms by which herpes simplex virus induces infection in the bodies of developing chick embryos following various methods of inoculation. In other words it has to do with pathogenesis, or the way in which the infectious agent goes about establishing disease in a susceptible host; and these experiments define various patterns of pathogenesis for herpes virus which follow different methods of inoculation into chick embryos. Special consideration is given to the involvement of endothelium and the dissemination of infection by way of the blood stream. In addition an increase of virulence of the HF strain for chick embryos will be briefly described, as well as a modification of the pathogenicity of the HF strain of virus for rabbits which occurred during serial membranal transmission.

Experimental Procedures

The strain of herpes virus used for these experiments was the HF virus (Rockefeller Institute, New York) whose encephalitogenic property for rabbits is well established. The technic used for exposing embryonic membranes has been described by Goodpasture and Buddingh.[7] A membranal strain of HF virus was easily established by inoculating the chorioallantois of 12 or 13-day old embryos with macerated infected rabbit's brain. Transfers were made by rubbing bits of infected membrane over the exposed surface of other membranes. Twelve and 13-day old embryos were used almost exclusively. A strain of virus thus established has been maintained for 2 years by means of 125 membranal passages uninterrupted except by irregular intervals of storage at 0°–4° C. Of chief importance in these experiments has been the microscopic examination of pathological lesions occurring in 256 autopsied embryos. Gross lesions were routinely noted. It is worthy of emphasis that except in extremely rare instances only live embryos were fixed for histological study. Whole embryos with split skulls and abdomens were fixed in Zenker's fluid (plus 5 per cent acetic acid), and sections for embedding were cut through the embryo perpendicular to the long axis of the body. The hematoxylin-eosin stain has proved en-

tirely satisfactory for the demonstration of herpetic inclusions. The presence of intranuclear inclusions characteristic of herpetic virus has been the rigid criterion for the recognition of infection in fixed tissue.

Infection of the Chorioallantois Following Membranal Inoculation

Although the lesions of the first membranal generations were complicated by the presence of macerated rabbit's brain, they were essentially characteristic. Small opaque foci of infection were recognizable 48–72 hours after inoculation. Of primary importance in microscopic sections is the presence of intranuclear inclusions in ectodermal and mesodermal cells of the membrane. Secondary to the presence of these inclusions is proliferation of ectoderm and mesoderm accompanied by a mild inflammatory reaction. As the lesions progress ectodermal cells tend to become necrotic and to desquamate. Depending on the severity of the infection healing may or may not follow. As serial passages multiplied, the severity of the membranal lesions increased but not in exact direct proportion. For example, the 2nd and 3rd generation lesions were confluent but relatively superficial and proliferative. After the 25th generation the membranal infection caused more destruction than proliferation, and since the 50th generation there has been little change in the character of the lesion. At about the 30th passage the ectoderm over the infected area was being entirely destroyed at 72 hours; there was deep involvement of the mesoderm, and it was grossly and microscopically evident that the circulation was severely interrupted. Petechial hemorrhages and thrombosis of small vessels were present.

Membranes of 9 through 19-day old embryos were found to be susceptible to infection with herpetic virus but the membranal response to infection is different at various stages of development. That the gross membranal lesions at a given hour interval vary with the age of the embryo has been observed by Schaffer and Enders,[5] but they seem doubtful about the characteristic nature of the gross and microscopic membranal reaction to herpes virus. In my experience membranal reactions to the virus of herpes, vaccinia, smallpox, fowlpox or laryngotracheitis are decidedly different when examined either grossly or microscopically at equal

intervals of time after inoculation of comparably aged embryos, but these differences cannot replace the specific cellular inclusion as criteria for the diagnosis of the virus concerned.

Membranes inoculated on the 17th day of incubation and younger develop grossly recognizable herpetic lesions. Gross lesions did not develop after the inoculation of 18 and 19-day membranes. Microscopically herpetic inclusions in ectoderm and mesoderm were regularly demonstrable in significant numbers for 4 or 5 days following inoculation of all membranes from 9 through 16 days incubation. Inclusions developed abundantly in the ectoderm of 17-day membranes but the reaction to infection was purely proliferative without necrosis or spread of the virus to the underlying mesoderm. This would indicate a developmental resistance of membranal ectoderm to infection. Membranes inoculated on the 18th or 19th day of incubation occasionally showed inclusions in ectodermal cells. Two factors must be considered in an analysis of the failure of 18 and 19-day membranes to develop appreciable lesions: one is that the blood at this stage is receding from active circulation through the membrane and the chorioallantois is becoming keratinized and dry in preparation for hatching; the other is that with maturation of the embryo factors of natural immunity which make adult fowls resistant to herpetic infection may be developing.

Infection of the Embryo Following Membranal Inoculation

Brain: From the 1st passage, embryos have been examined for metastatic lesions. Of 28 embryos inoculated on the chorioallantois with infected rabbit's brain, *i.e.* the first membranal generation, only 5 have shown metastatic lesions. Examined microscopically 2 embryos of this series showed an herpetic encephalitis 7 days after inoculation. In 1 embryo the encephalitis was extremely extensive and destructive. A large portion of the brain was necrotic. Most of the remaining neurons, glial cells and ependymal cells contained large intranuclear inclusions characteristic of herpes. There was no evidence of meningitis or myelitis, nor was there any appreciable inflammatory response. The other embryo showed neither hemorrhage nor widespread necrosis. Neurons and glial cells in a localized area contained typical inclusions. It may be of some significance that metastatic en-

cephalitis has been observed only in 1 embryo (3rd passage) beyond the 1st generation.

Heart: Two other embryos inoculated with rabbit's brain showed an herpetic myocarditis. One was fixed 6 and the other 7 days after membranal inoculation. The infected hearts were greatly enlarged and in microscopic sections the myocardial cells in the infected foci are pale staining and are swollen and without evident striations. The nuclei of the cells are enlarged, chromatin is condensed at the nuclear wall, and as in cells of any tissue the inclusions may vary from small condensed acidophilic bodies surrounded by a clear zone to large granular basophilic masses which fill the whole nucleus. The degree of inflammatory reaction appears to vary with the amount of necrosis produced by the infection. Only the lesions in the myocardium appeared repeatedly during the early serial passages and then only after 6 or 7-day membranal infection. After the 10th passage in one series gross cardiac lesions were consistently observed.

Liver: The other 1st passage metastasis was to the liver of a single embryo inoculated on the 11th day of incubation. Only three or four cells in one section were observed to contain inclusions. By the 15th passage the liver was regularly enlarged and showed multiple gross lesions at 5 and 6 days. In another series of passages the virus was not observed to metastasize either to the heart or liver until after the 20th generation. In the latter series the heart was not involved until after the liver began to show gross lesions regularly 5 days after inoculation. Between the 30th and 40th passages of the virus in both series all embryos autopsied 96 hours after inoculation showed enlarged livers dotted with multiple foci of necrosis. The hearts were edematous, pale and flabby with large yellowish plaques in the myocardium. The pericardial sacs and peritoneums were usually filled with a cloudy yellowish fluid.

Microscopically the hepatic lesions vary with the age of infection. At 48–72 hours parenchymal and stromal cells in multiple foci contain inclusions. The infection spreads until sometimes the lesions become confluent and practically every cell in the liver is involved. Necrosis begins at the center of a focus. Actual vesiculation occurs within the liver foci but there is very little cellular response.

Spleen, Kidney, Lung and Other Organs: The spleen has regularly shown gross as well as microscopic lesions but with reference to serial passage they have appeared later than the hepatic and myocardial lesions. The stromal cells in the spleen are the first to develop inclusions. They are small and brightly eosinophilic. The infection spreads and necrosis and vesiculation follow, as in the liver.

Microscopic lesions in the lung, kidney, smooth and striated muscle, subserosa of the stomach and intestines, periosteum, and infection of osteoblasts and chondroblasts were observed repeatedly in microscopic sections. In the lung and subserosa, as in the spleen, connective tissue cells appear to be the first involved and lesions in these organs remain localized. In the kidney the chief lesions are in the glomerular tufts. The infection is bilateral. Both epithelial and endothelial cells of infected tufts are enlarged and contain typical inclusions; necrosis and a mild inflammatory reaction develop secondarily. Well localized interstitial lesions were occasionally observed and in a few instances cells of Bowman's capsule and collecting tubules contained inclusions. From the distribution, frequency, and general character of lesions in the glomeruli, the glomerulonephritis seemed to develop from an initial infection of glomerular endothelium.

Although the mesonephros and the metanephros were both susceptible to infection the mesonephric glomeruli were by far more frequently and extensively involved. It is possible that this is in some way related to the fact that at this stage in development the mesonephros is the more active kidney and is carrying the burden of embryonic excretion.

Endothelium: Of particular interest has been the observation of typical intranuclear inclusions in the endothelium of blood vessels. In an embryo inoculated with virus of the 38th generation and sacrificed after 96 hours the endothelium of the aorta and of a large mesenteric artery was found to contain inclusions. Infected cells were round and slightly enlarged, and many appeared desquamated. The smooth muscle of the media about the intima of the mesenteric vessel was extensively involved. In other embryos the endothelium of the capillaries in the heart, liver and muscle was found to contain inclusions, as were the endothelial cells of the glomerular tufts of the kidney.

Pathogenesis of Embryonic Infection Following Membranal Inoculation

Up to this point all evidence pointed toward the spread of infection from the chorioallantois to various organs of the embryo by way of the blood stream. To test this hypothesis 2 experiments were carried out. Membranes of 12 12-day embryos were inoculated with the 61st generation of chick virus and 12 other embryos received macerated rabbit's brain infected with the original HF virus. At 24-hour intervals 2 embryos from each lot were bled aseptically from the allantoic artery where it leaves the umbilical stalk. Blood from each embryo was immediately inoculated in 0.1 cc. quantities onto other 12-day membranes. After incubation for 72 hours these membranes were fixed. Herpetic lesions were grossly demonstrable in most cases. Microscopically it was determined that the 61st generation virus had been consistently present in the blood stream at 24, 48, 72, 96 and 120 hours after inoculation. The original HF virus was recovered from the circulating blood in 1 instance at 48 hours and regularly at later intervals.

Adult chickens were hyperimmunized, by repeated intravenous injections, with the passaged strain of virus. Serum was collected and injected intravenously in 0.15 cc. amounts into 12 and 13-day old embryos. Within 2 hours after the injection of antiserum membranes of 10 test embryos and 12 control embryos were inoculated with the 107th generation of virus. Deaths of 4 test embryos within 24 hours were attributed to shock or hemorrhage. Each control membrane developed a typically spreading destructive lesion. All embryos of this group except 1 died on the 4th day. Membranes of the test embryos developed well circumscribed, thick purulent lesions with isolated pox at the periphery. All 6 were alive and vigorous at 96 hours. Autopsied embryos of the control group showed marked enlargement and extensive necrosis of the heart and liver with an abundance of cloudy peritoneal and pericardial fluids. Of the test group 1 showed no macroscopic lesion at all in the heart or liver; 4 showed only three or four foci of necrosis in the liver with no enlargement of the liver or heart; and the other showed multiple foci of necrosis in the liver without involvement of the heart or an accumulation of fluid. Microscopically the herpetic lesions of these embryos are small,

definitely circumscribed, and only a very few inclusions can be found. Thus by passive immunization embryos were protected against a lethal herpetic infection; the lesion at the site of inoculation was markedly modified; and metastatic spread of infection was largely prevented. One explanation might be that virus which gained entrance to the blood stream was there neutralized by circulating antibodies passively introduced. To say the least, the modification of the spread of infection by passive immunization adds evidence to the hypothesis of a hematogenous spread of infection.

In an effort to reconstruct the mechanism for the development of a systemic herpetic infection in chick embryos, one recognizes the local multiplication of virus in cells at the site of inoculation on the chorioallantois. The susceptibility of endothelium to infection makes the vascular channels vulnerable to invasion by the virus. The virus can be recovered from the circulating blood or can be neutralized within it by passive immunization. Again the susceptibility of embryonic endothelium to herpetic infection affords a means of establishing a metastatic lesion in endothelium and subsequent spread from endothelium to contiguous susceptible tissues. The demonstration of herpetic inclusions in endothelium makes such an explanation tenable.

One is confronted then with a difficulty in explaining not only quantitative but qualitative differences in the spread of infection during very early and later membranal passages. During serial passage the pathogenicity of the virus becomes enhanced for mesodermal tissue which is inclusive of endothelium. This may account for the greater number of visceral lesions of later generation, but one must look elsewhere for an explanation of the failure of the virus to localize in the brain after its pathogenicity for endothelium has become enhanced.

Infection Following Subamniotic Inoculation

Polk, Buddingh and Goodpasture [8] and Buddingh and Polk [9] described the practicability of infecting chick embryos by making injections of infectious agents into the amniotic sac. In the following experiments 13-day old embryos were inoculated into the amniotic cavity with 0.1 cc. of a 1:10 dilution of ground membranes infected with the 10th generation of chick virus. (In this

series the virus did not metastasize to the liver and heart until after the 10th generation.) Embryos were fixed at 24-hour intervals through 96 hours. At 72 hours the amnion was thickened and the skin of the embryo appeared more opaque and less smooth than normal. Microscopically the ectoderm, mesoderm and muscle cells of the amnion contained herpetic inclusions at 48 hours, as did the epithelium of the skin. Proliferation of cutaneous epithelium at a focus of infection was followed by vesiculation at 72 hours and ulceration at 96 hours. Similar lesions developed at multiple foci in the epithelium of the pharynx. Presumably by direct extension of infection an herpetic peritonitis developed regularly. In a few cases infection extended from visceral peritoneum to liver cells and to the cortex of adrenals.

Attempts to repeat this experiment with virus past the 50th generation have been rather unsatisfactory. Infection of the amnion led to a rapid dissemination of virus by the blood stream and early death of the embryos. Lesions in the pharynx were not observed and the skin of only 1 embryo was infected.

Infection Following Scarification and Inoculation of Skin

A series of 13-day embryos was inoculated on scarified skin of the thigh with 1:10 dilution of ground 10th generation membranes. Working with small forceps through a slit in the chorioallantois the thigh was gently manipulated and steadied so that a needle inserted beneath the amnion could be used to scarify and inoculate the skin with one procedure. Embryos were fixed at 24-hour intervals through 72 hours. Areas of necrosis at the site of inoculation appeared grossly. Microscopically there was extensive necrosis of epithelium and subcutaneous tissue. Intact epithelium and underlying corium were infected. Epithelial hyperplasia resulted in the formation of discrete papules in which all cells contained inclusions. By direct extension the virus invaded striated muscle beneath the superficial lesion. The nucleus of every muscle cell in a limited area contained an inclusion. An herpetic peritonitis developed in these embryos.

Infection Following Subcutaneous Inoculation

Without manipulation 13-day old embryos were inoculated subcutaneously with 0.05 cc. of 1:10 suspension of 10th genera-

tion membranal material. Embryos were fixed at 24-hour intervals through 96 hours. Gross lesions developed at the site of injection. Microscopically these lesions were comparable to those which followed scarification: epithelium, underlying connective tissue and muscle cells contained inclusions. There was an herpetic peritonitis. Remarkable in 1 embryo fixed at 96 hours was the occurrence of a destructive ascending myelitis following subcutaneous inoculation lateral to the dorsal midline at the lower sacral level. In this case there was a specific lesion at the site of injection and a mild peritonitis. The spinal cord at the level of inoculation showed a lesion which was definitely more severe on one side than on the other. Both supporting cells and neurons contained well defined inclusions. At the lumbar level the myelitis was uniformly bilateral and extremely destructive. Practically every cell of the cord in cross section was either necrotic or infected. As the infection ascended it remained bilateral but lessened in intensity and progressed more rapidly in the ventral than in the dorsal columns. At this 96-hour interval the infection had not reached the brain.

Pathogenesis of Embryonic Infection Following Subamniotic and Subcutaneous Inoculations and Inoculation After Scarification of the Skin

The mechanism of the infectious process following these three methods of inoculation is essentially the same. In each case virus is introduced into amniotic fluid and is in direct contact with amnion and cutaneous epithelium. Amniotic fluid is aspirated to bring virus in contact with pharyngeal epithelium. Each epithelial surface involved is susceptible to virus brought in contact with it. Infection is initiated by direct contact and spreads from cell to cell along the epithelial surface and into adjacent tissues of a different type. The herpetic peritonitis likely results from direct extension of infection from the amnion, although the possibility of a primary peritoneal infection following incidental introduction of virus into the communicating extra-embryonic coelom must be considered. Infection of nerves at a local focus will be considered in another connection. The fact that epithelial cells are not readily attacked by virus of the later passages suggests the possibility of a loss of some degree of ectodermotropism.

Infection Following Intracerebral Inoculation

With careful candling eggs were opened over the heads of a series of 13-day embryos and inoculations were made directly into brains without manipulation of the embryos. Five hundredths of a cc. of 1:10 suspension of 10th generation membranal material was injected. Embryos were fixed at 24-hour intervals through 72 hours. At 24 hours there was a localized meningitis at the site of injection and a local infection of the brain. After 72 hours an extensive encephalomyelitis had developed. There were large areas of hemorrhage and necrosis in the cerebrum. Neurons, glial and ependymal cells contained inclusions. All parts of the brain appeared invaded. The infection was generally destructive as it descended the cord but diminished in intensity at the lower levels. As in the ascending myelitis the progress was more rapid in the ventral columns. In the lumbar region a section containing kidney, intestine and gizzard showed that the infection had progressed peripherally by way of the ventral horn cells through the ventral roots to the spinal nerves. Serial sections were studied and by the occurrence of intranuclear inclusions in cells of the perineurium the peripheral spread of infection was followed. Peripheral extension was bilateral through the spinal roots. Neurons of the dorsal ganglia and the dorsal tracts of the spinal nerves did not appear infected. Spread along the somatic branches of the spinal nerve was followed to small radicals of nerves in the striated muscle of the body wall. Infection of these muscles was not observed. Extension of infection along the splanchnic branch to the sympathetic system could likewise be followed. Most of the cells of the sympathetic ganglia at the surface of the gizzard contained inclusions. Perineural cells of smaller radicals and small groups of neurons deep within the gizzard were infected. Here the smooth muscle cells themselves contained inclusions. Ganglion cells about the intestines were also involved in the peripheral spread of infection. In this case there were no lesions in the liver, heart, lungs or kidneys.

Efforts to repeat this experiment by inoculating the 68th generation of the virus directly into the brain were complicated by the fact that virus from incidental membranal lesions rapidly invaded the liver, heart and lungs by the blood stream. The lesions in the

brain appeared less severe. In 1 embryo there was a severe meningitis with a relatively mild lesion in the brain. No destructive myelitis was induced by the 68th generation virus.

Pathogenesis of Infection Within the Nervous System

Transmission of herpes virus from cell to cell along peripheral nerves and within the central nervous system of chick embryos is recognized. Once cells of a nerve or of the cord and brain become infected extension of infection appears to be directed along cells of the nervous system without involvement of adjacent tissues, but these experiments present no evidence of a restrictive spread among particular cells within the nervous system. Cells of the perineurium, glial cells and ependymal cells, as well as neurons, were involved in the spread of infection. A more sensitive susceptibility of neurons is suggested by a more rapid spread along motor neurons within the cord. In another instance the neurons in sympathetic ganglia appeared to be predominantly involved in a centrifugal spread of infection from the cord.

There is no indication that endothelium becomes infected within the central nervous system or that a hematogenous metastasis has resulted from a primary infection of the brain. Virus of later passages, which is judged to possess an enhanced pathogenicity for endothelium, has not localized in the brain from the circulating blood. On the other hand, virus not infrequently localized in the brain from the blood stream during very early passages. It is questionable whether one can adequately explain these two divergent behaviors of virus within the brain on the basis of a quantitative reduction of neurotropism.

Modification of the Virus During Serial Passage

Certain modifications in the behavior of the virus have occurred during membranal passage. An increase in virulence of the passaged strain for embryos is indicated in the foregoing experiments. As the passaged virus became more virulent for chick embryos gross lesions appeared at earlier intervals, and more extensive metastasis and more rapid spread occurred. Twelve or 13-day old embryos inoculated with original HF virus would usually survive 7 days. Embryos inoculated with the 125th generation virus of Series I died regularly at 96 hours. Infection with the

50th generation virus of Series II killed all embryos during the 3rd day. In terms of tissue affinities the virus may be said to have become increasingly mesodermotropic. This tropism is expressed by a more facile infection of endothelium, smooth muscle and connective tissue. Endoderm as represented by liver cells is more readily infected after serial passage of the virus on membranes. Although the present experiments are not conclusive, the possibility of a reduction in virulence for cutaneous ectoderm and pharyngeal epithelium is suggested; also a possible reduction in neurotropism is evidenced by failure of the virus to localize in the brain during late passages and a less rapid spread within the central nervous system after direct inoculation.

As previously reported, the pathogenicity of the HF virus for rabbits became in Series I suddenly modified during membranal passages.[10] After the 20th membranal generation of the 1st series of passages the chick strain has not induced an encephalitis in rabbits following scarification and inoculation of the cornea. It was not possible to recover the virus from the gasserian ganglia or from the brain at the entrance of the fifth nerve 4 and 7 days after inoculation of the cornea. When inoculated intracerebrally into rabbits the original HF virus was infective in higher dilutions than the chick strain. Further consideration of this striking modification is outside the scope of this report. Attempts to induce a similar modification by serial passage of the same (HF) strain have not yet succeeded.

Studies of the modification of the pathogenicity of the passaged virus for hatched chicks are fragmentary. Suffice it to say that in 2 experiments chicks 2 or 3 weeks old developed focal areas of necrosis in the liver following intraperitoneal inoculation with the passaged virus. These focal lesions appeared much like the focal lesions in embryonic livers but inclusions could not be demonstrated. At the present time the strain's pathogenicity is not judged to be so modified as to induce evident infection in hatched chicks.

Discussion

The experiments described above present three mechanisms by which herpes virus has induced extension of infection in chick embryos.

(1) Virus in contact with intact uninjured embryonic epithelium invaded epithelial cells and apparently multiplied within them. Intranuclear inclusions developed along with other cellular changes. There was a stimulus to epithelial hyperplasia. By some means as yet obscure the virus spread laterally to other epithelial cells and vertically to contiguous cells of different tissues. Proliferation, mild inflammation, necrosis, vesiculation and desquamation are parts of the pathological process. This mode of pathogenesis operates to establish infection of the membrane following inoculation of the chorioallantois. It is the essential mechanism of cutaneous and pharyngeal as well as amniotic and peritoneal infections following inoculation into amniotic fluid, because virus in amniotic fluid is in contact with the skin of the embryo, is aspirated into the pharynx, and spreads by extension of infection to peritoneal mesothelium.

(2) The virus may invade the blood stream at the site of a local lesion and be disseminated to establish a generalized herpetic infection in the embryonic host. Invasion of the blood stream was established by recovery of the virus from the circulation. The ability of circulating virus to initiate metastatic lesions was evidenced by the demonstration of intranuclear inclusions in endothelium of both large blood vessels and capillaries. It seems that by successive membranal passage the virus becomes more virulent for endothelium and other mesodermal elements, such as cardiac muscle, as well as for cells of the liver. Further evidence of the infectivity of blood-borne virus is that metastatic lesions may be prevented partially or completely in embryos receiving an intravenous injection of herpetic antiserum. Since the virus is able to enter and escape from the circulating blood the occurrence of metastatic lesions in other organs may be governed to some extent by the susceptibility of the cells of those organs. From these experiments it appears that the parenchyma of the liver, heart muscle and connective tissue cells is most easily invaded. Infections in the heart and liver spread so rapidly one cannot judge whether parenchyma or stroma is first involved. Blood from the chorioallantois of 12-day embryos passes first through the sinuses of the liver and then to the heart before systemic distribution is effected. Thus if the primary lesion is in the chorioallantois the highest concentrations of circulating virus should enter the

liver and the heart. This may explain the early and extensive involvement of these organs rather than a greater susceptibility of these types of cells. The cells of the interstitial tissue of the lungs, the stroma of the spleen and subserosa of the gastrointestinal tract are the first to develop inclusions in these organs. A single stromal cell was often observed to contain a brightly acidophilic inclusion before spread, proliferation or an inflammatory reaction had occurred. The herpetic glomerulonephritis appears to represent hematogenous metastasis to the glomerular tufts. Whether the lesions in the tubules are of hematogenous or of urinary origin has not been determined histologically.

A consideration of the localization of virus in the brain from the blood stream presents several problems not fully answered by these experiments. The fact that virus localized in the brain in 2 of 7 embryos allowed to go 7 days following membranal inoculation with infected rabbit's brain may be significant. In only 1 other embryo (3rd passage, 7-day infection) was such localization observed. It may be more significant that the virus has not localized in the brain as serial passages have increased. It must be kept in mind that in later generations embryos do not live 7 days after being inoculated and time may be an important factor in the development of the brain lesion. These experiments suggest that the original HF virus and virus of very early membranal generations possess a neurotropism which diminishes for the chick embryo during repeated membranal passage, but no adequate explanation seems to be at hand to explain the mode of infection of the brain in 1st and early passages. On the other hand, during prolonged cultivation of the same strain of virus on the membrane in the absence of any neural elements it is apparent that it has become increasingly mesodermotropic for embryonic tissues and it is not incompatible with the evidence of these experiments to conceive of a concurrent loss of a high degree of affinity for nervous tissue.

(3) The third mode of pathogenesis presented by these experiments is a neural transmission of virus in chick embryos. Both centripetal and centrifugal progress of herpes virus within the central nervous system are illustrated; the one by an ascending myelitis from the sacral level of the cord, the other by a descending encephalomyelitis following an intracerebral inoculation. Proof of axonal transmission along the peripheral nerves to and from

the central nervous system is complicated by the fact that the perineural sheaths of spinal nerves and their radicals are susceptible to herpetic infection. Since perineural cells arise from the same germinal layer as the neurons the same tissue affinities may govern passage along these sheaths as from axon to axon. However, spread within the central nervous system is probably by way of axons. In the cases cited the other two modes of pathogenesis, namely by surface contact and by way of blood transport, can be reasonably ruled out. In neither case is there any metastasis to the liver or heart which would indicate either total absence of virus from the blood stream or its presence in subinfective quantities. In the embryo inoculated subcutaneously there was a mild local lesion at the site of injection with no evidence that the virus had reached the spinal cord by indiscriminate spread from cell to cell. Yet in the cord at the sacral level there was infection which is judged to have resulted from centripetal spread of the virus along the nerve and its sheath from the local lesion. In the other instance centrifugal spread from brain to cord following intracerebral inoculation is obvious and the infection extended bilaterally from the cord by the ventral roots to the spinal nerves. By infection of consecutive sheath cells extension to radicals in striated muscle of the body wall could be followed. In like manner spread by the sympathetic nerves to the smooth muscle in the gizzard was established. The fact that neurons in the gizzard contained inclusions points to spread along the axons themselves.

In considering these different modes of pathogenesis of herpetic infection in chick embryos one becomes aware both of similarities and of dissimilarities to herpetic infection in other animals. For example the vesicular lesions in the skin and pharynx of embryos are comparable to the skin lesions of herpes febrilis and herpetic stomatitis in human beings. Actual vesiculation of lesions in the liver and spleen emphasizes the ability of the virus to stimulate consistently similar reactions. Neural transmission of the virus follows a principle well established in rabbits. On the other hand the presence of herpetic inclusions in vascular endothelium has not to my knowledge been previously described, nor has blood-borne metastasis been definitely established. Herpes virus is rarely demonstrable in the circulating blood of man with natural infec-

tions nor is it usually recoverable from the blood of experimental animals with induced infections. In the experience of Goodpasture and Teague [11] the injection of large amounts of virus intravenously into rabbits was necessary to induce an occasional localization of virus in the brain; and cells of the liver, adrenal and testes in rabbits developed inclusions only if they were injured and locally inoculated. Although this hematogenous potentiality of herpes virus is not usually expressed by herpetic infection in other animals, it is comparable to the behavior of certain other neurotropic viruses. For example, yellow fever virus is known to circulate in the blood stream and to localize in the liver; and the virus of canine distemper manifests an affinity for vascular endothelium and will localize in the liver as well as in the brain of dogs and ferrets.[12] Sabin describes metastases of B virus from the testes to the liver, spleen and adrenals of rabbits [13]; and Burnet, Lush and Jackson observed lesions in the livers of chick embryos following membranal infection with B virus.[6] However, there is no evidence that herpes virus modified by embryonic passage has acquired the property of inducing blood-borne infections in rabbits analogous to virus B.

Certain modifications in the behavior of herpes virus have been observed during serial membranal transfers. The increase in virulence for embryos, as evidenced by a wider dissemination and a more rapid spread of infection, as well as by an enhanced lethal effect, is in keeping with the behavior of many other infectious agents during repeated passage through a single host specie. However, the fact that during serial passage in chick embryos this highly encephalitogenic strain of herpes has lost its ability to induce an encephalitis in rabbits following inoculation of the cornea is of considerable interest. During propagation on an avian host a modification in pathogenicity for a mammalian host has occurred. The nature, stability and immunological aspects of this change will be considered in a subsequent report. Suffice it to point out that modification of an avid neurotropism has occurred. Such modifications of neurotropic viruses might be of importance from the standpoint of practical application to vaccination. However, investigators must be continually aware that undesirable modifications might occur under the same set of circumstances and this emphasizes the importance of adequate histological and

biological controls on the behavior of viruses maintained in an experimental environment.

Summary

1. Three modes of pathogenesis of herpes simplex virus in chick embryos have been described: (1) invasion of epithelium by direct contact and spread by continuity of infection; (2) hematogenous transmission of the virus to establish a generalized herpetic infection; and (3) both centripetal and centrifugal neural transmission of infection.

2. Endothelial cells of large blood vessels and of capillaries of chick embryos were found to contain herpetic inclusions indicating a mode of localization and of metastases through established endothelial foci.

3. Embryonic chick cells of skin, pharynx, peritoneum, amnion, liver, heart, smooth and striated muscle, lung, spleen, kidney, adrenal, subserosa of gastro-intestinal tract, and the central and peripheral nervous system, as well as osteoblasts and chondroblasts, were found to be susceptible to herpetic infection.

4. An increase in the virulence of the virus for chick embryos is expressed by an enhancement of its ability to infect mesodermal and certain endodermal tissues.

5. Evidences of a diminution in ectodermal and neuronal tropisms are presented.

6. During serial membranal transfer the HF strain of herpes virus has lost suddenly on one occasion its ability to induce an encephalitis in rabbits following corneal inoculation. The conditions of this change are not known, and thus far are not repeatable at will.

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DESCRIPTION OF PLATES

PLATE 41

FIG. 1. Gross appearance of infected embryonic chick organs. "A" shows enlargement, multiple foci of necrosis in the liver and spleen, and plaques of necrosis in the heart, as compared with normal organs "B." 38th passage, membranal inoculation, 6-day infection.

FIG. 2. Lobe of an embryonic chick liver showing the extent and distribution of focal metastatic infection with herpes virus. 75th passage, 96-hour infection. × 14.

FIG. 3. Single focus of infection in the liver spreading from a blood vessel. Central necrosis, only slight leukocytic infiltration, and abundance of inclusions in the liver cells at the periphery of the lesion. 81st passage, 96-hour infection. × 120.

FIG. 4. Vesiculation within a single hepatic lesion. Fibrin and leukocytes are present in the area of liquefaction. Narrow zone of necrosis. The inclusions are present in a peripheral zone. 75th passage, 96-hour infection. × 325.

FIG. 5. Two intranuclear inclusions of herpes in parenchymal cells of the liver. One shows an acidophilic inclusion surrounded by a clear zone, with condensation of chromatin at the nuclear wall; the other shows a slightly basophilic granular mass filling the whole nuclear space. 81st passage, 96-hour infection. × 1100.

FIG. 6. Seven intranuclear inclusions of herpes in parenchymal cells of the liver. 81st passage, 96-hour infection. × 1100.

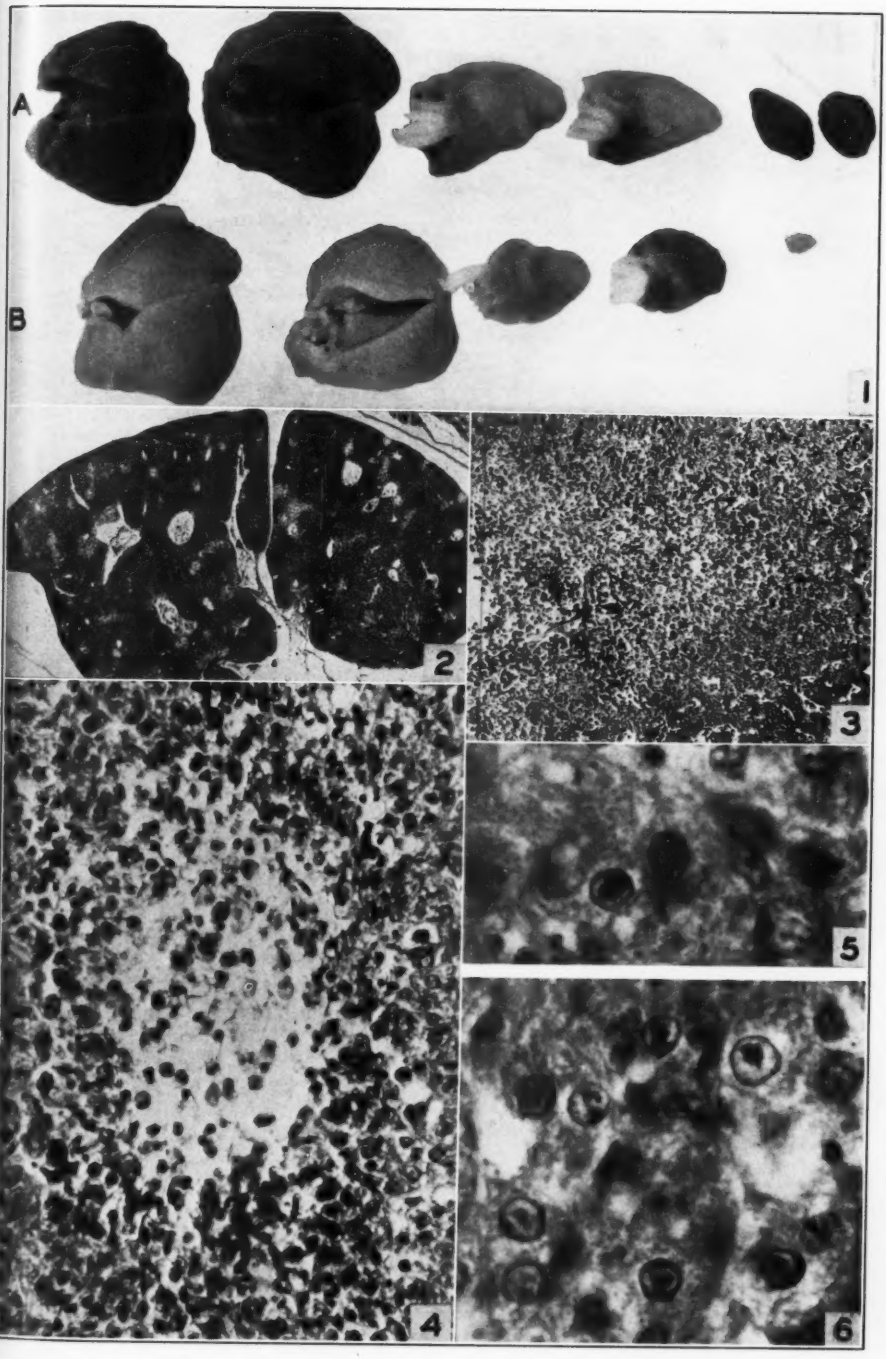
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PLATE 42

FIG. 7. Cross section of an embryonic chick heart showing the focal nature and distribution of herpetic metastases to the myocardium. 23rd passage, 6-day infection. × 18.

FIG. 8. Higher power of the central focus of infection shown in Figure 1. Note the central zone of necrosis with but slight infiltration of leukocytes, and the pale peripheral zone of swollen muscle cells where herpetic inclusion are most numerous. 23rd passage, 6-day infection. × 80.

FIG. 9. High magnification showing specific intranuclear inclusions of herpes virus in three smooth muscle cells of the myocardium. Acidophilic inclusions surrounded by a clear zone with condensed chromatin at the nuclear wall are seen. 23rd passage, 6-day infection. × 1200.

FIG. 10. Four smooth muscle cells of the myocardium containing herpetic inclusions. 23rd passage, 6-day infection. × 1200.

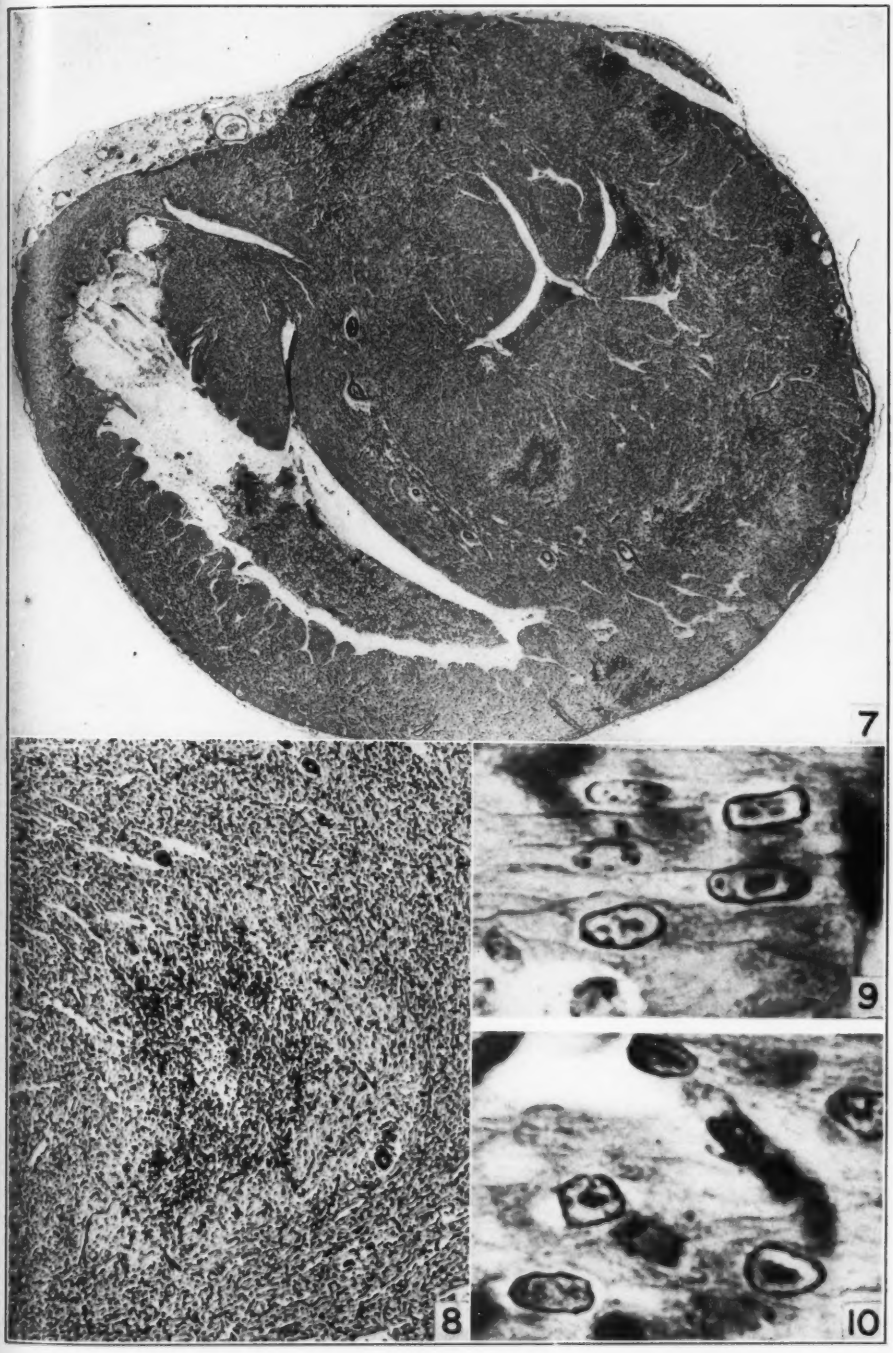
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PLATE 43

FIG. 11. Mesenteric artery of a chick embryo which showed herpetic inclusions in the endothelium and in the *tunica media*. 38th passage, 96-hour infection. × 120.

FIG. 12. Intranuclear inclusions in three endothelial cells from the blood vessel illustrated in Figure 11. 38th passage, 96-hour infection. × 1200.

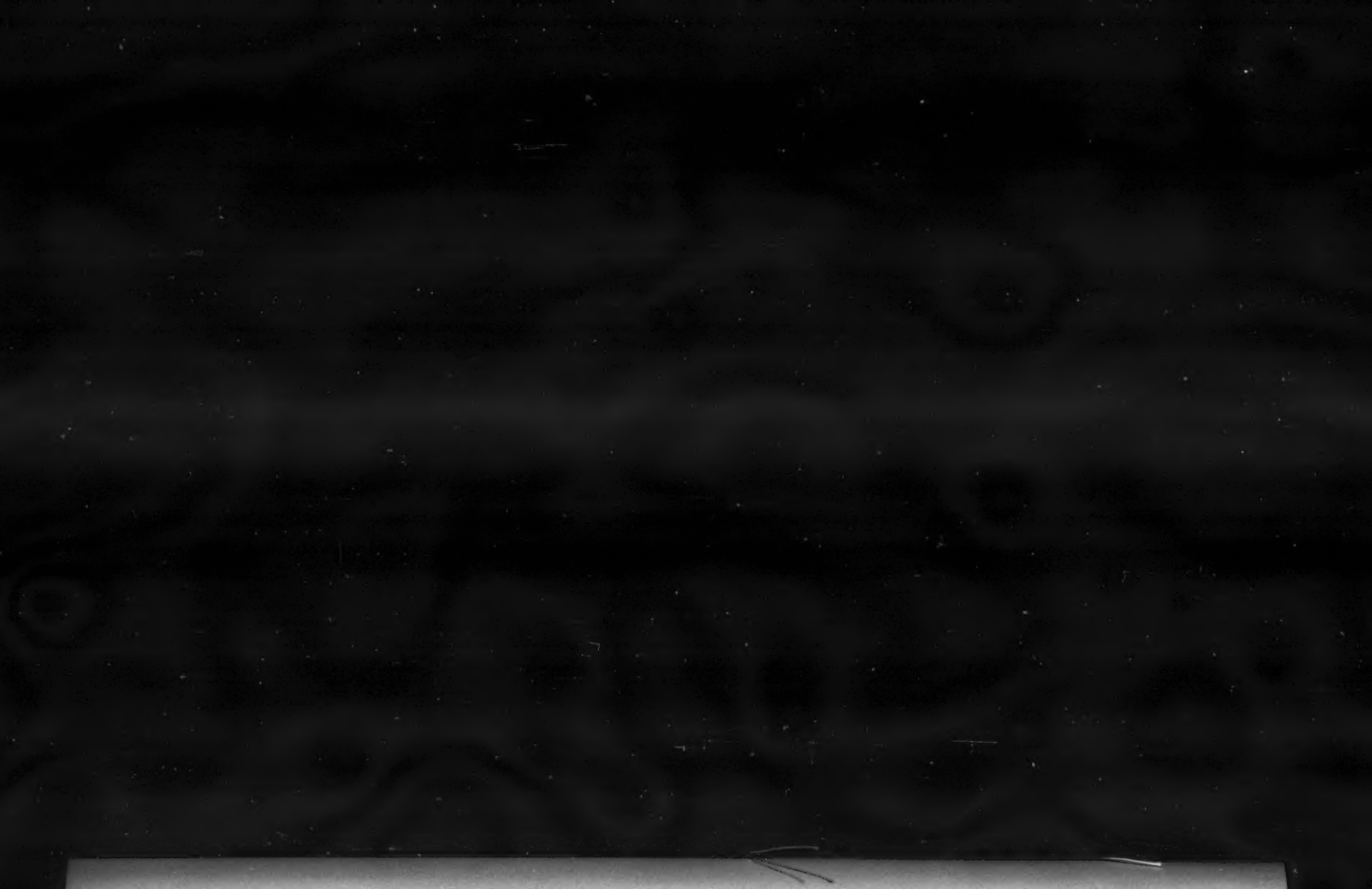
FIG. 13. Five inclusions in the *tunica media* of the blood vessel illustrated in Figure 11. 38th passage, 96-hour infection. × 1200.

FIG. 14. Herpetic inclusions in the endothelium and five infected smooth muscle cells from the blood vessel illustrated in Figure 11. Note that the endothelium has ulcerated and thrombocytes (black dots) are being deposited at the endothelial surface. 38th passage, 96-hour infection. × 1200.

FIG. 15. Intranuclear inclusions in three capillary endothelial cells in the liver (indicated by arrows). Note also the inclusions in the parenchymal cells both above and below the capillary. 81st passage, 96-hour infection. × 1100.

FIG. 16. Section showing the focal distribution of metastatic lesions in the spleen. 38th passage, 96-hour infection. × 38.

FIG. 17. Two metastatic foci of infection in the lung. 56th passage, 72-hour infection. × 38.



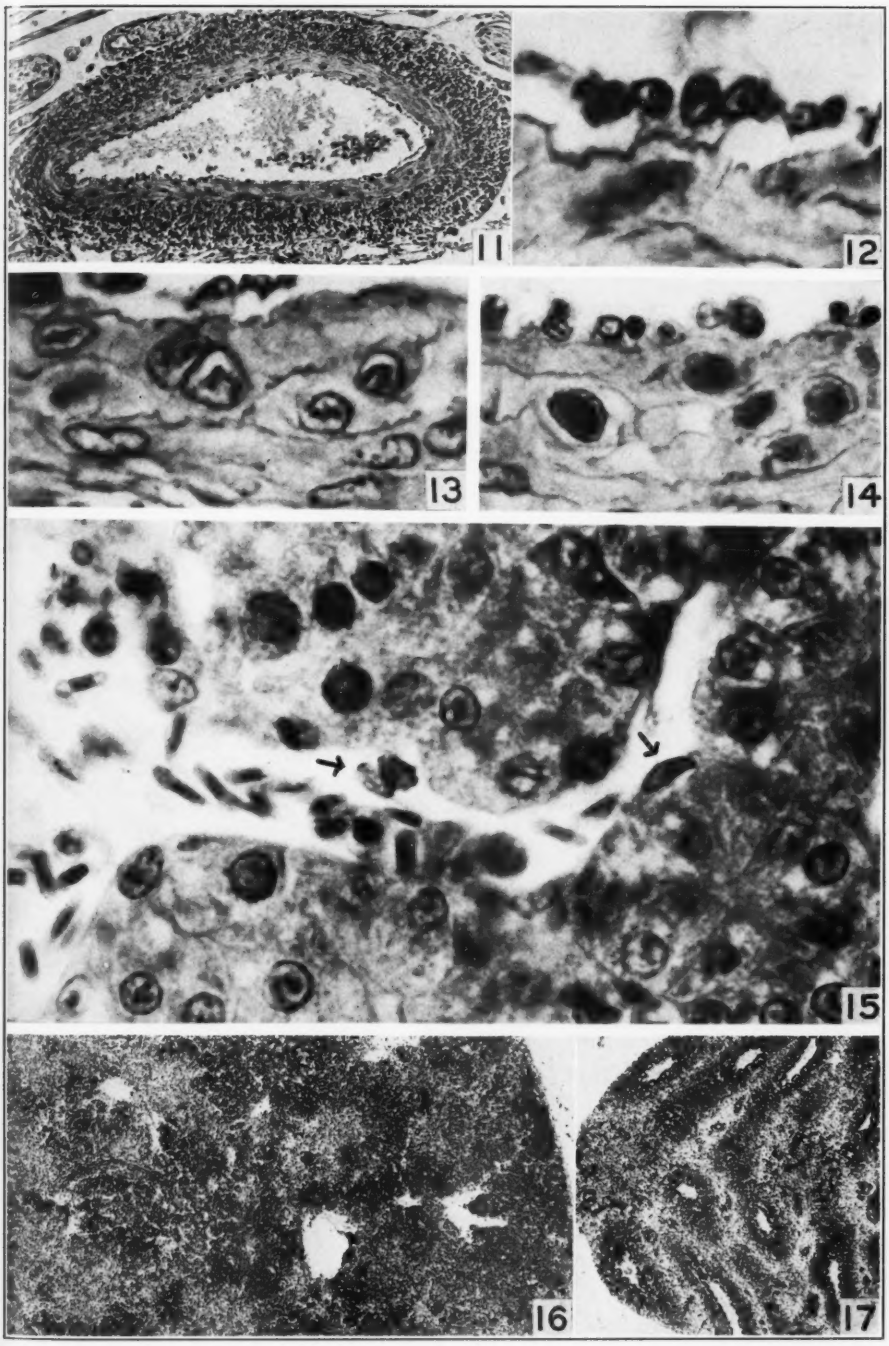

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PLATE 44

FIG. 18. Herpetic infection of a glomerulus. Note the central area of necrosis without appreciable infiltration of leukocytes. Inclusions are also visible within the glomerular tuft. 38th passage, 96-hour infection. × 525.

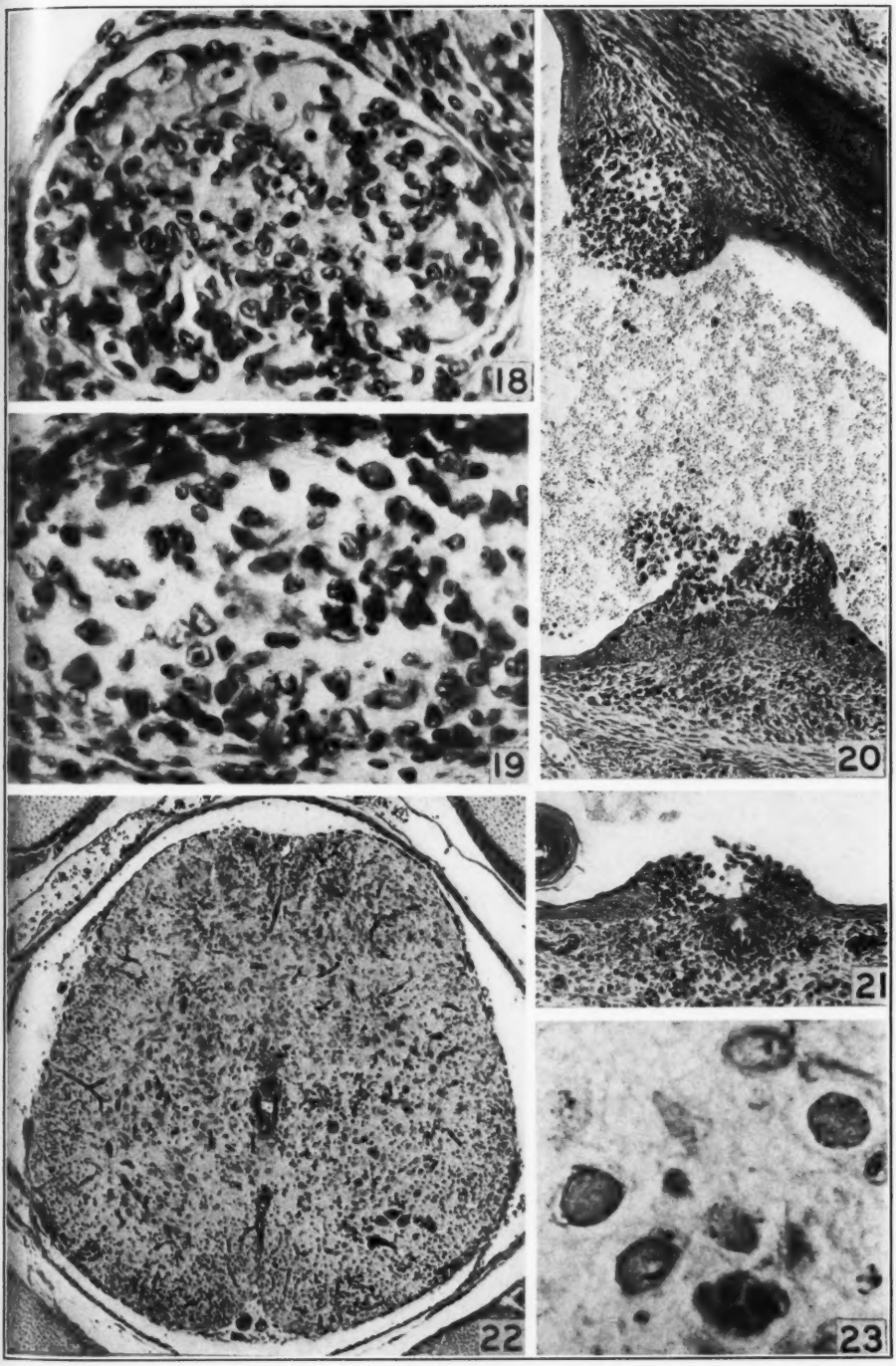
FIG. 19. Herpetic inclusions in the neurons, glial and sheath cells of a sympathetic ganglion at the surface of the gizzard. 38th passage, 96-hour infection. × 525.

FIG. 20. Herpetic lesions in the pharyngeal epithelium. Hyperplasia, vesiculation, ulceration and spread to underlying corium (upper lesion) are seen. 10th passage, subamniotic inoculation, 96-hour infection. × 120.

FIG. 21. Herpetic lesion in the cutaneous epithelium. Hyperplasia and vesiculation are seen. 10th passage, subamniotic inoculation, 96-hour infection. × 120.

FIG. 22. Cross section of the cord showing herpetic myelitis. Every motor neuron, many glial and ependymal cells are infected. A slight amount of hemorrhage and minimal inflammatory reaction are seen. 10th passage, subcutaneous inoculation, 96-hour infection. × 65.

FIG. 23. Herpetic inclusions in four neurons in the brain following intracerebral inoculation. × 750.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THE PATHOGENESIS OF SYPHILITIC OPTIC ATROPHY *

A Preliminary Study with Report of a Case

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The importance of syphilis as an etiological factor in the optic neuropathies is recognized by ophthalmologist, neurologist and syphilologist alike. Syphilis out-numbers all other causes combined in the etiology of primary optic atrophy.[1, 2] A review of the literature on the subject is contained in Moore's comprehensive monograph.[3] Much discussion is devoted to the various forms of syphilitic optic atrophies, and emphasis is laid on the difficulty in differentiating them either clinically or pathologically. It is well known that in some cases primary optic atrophy cannot be distinguished from that due to basilar meningitis. Igersheimer [4] pointed out in 1918 that syphilis may produce atrophy of the optic nerve in a variety of ways: (*a*) in association with disease of the bulb (the choroidoretinitis of congenital syphilis, or the intra-ocular inflammatory conditions of acquired syphilis); (*b*) in gumma or periostitis of the orbit, due to direct pressure on the intra-orbital portion of the nerve; (*c*) in basilar syphilitic meningitis, where the atrophy may be secondary, resulting from papillitis, or apparently typically primary; (*d*) due to pressure of syphilitic inflammatory products on the cerebral optic pathways anywhere from the orbit to the lateral geniculate bodies; and (*e*) as the genuine optic atrophy of tabes dorsalis, taboparesis and dementia paralytica.

With regard to the last mentioned form, it is important to emphasize the differences between the pathology of tabes and that of dementia paralytica. In the latter, spirochetes have been found in great numbers in the nervous tissue, and also in the meninges and about the blood vessels. There is actual destruction of brain cells, but cranial nerve palsies are rare and optic atrophy is uncommon unless tabes also exists. The primary lesion in tabes is probably not within the central nervous system at all, but in

* Received for publication March 11, 1939.

the posterior roots. There are several theories summarized in 1929 by Stern [5] as to the exact location of the lesion and the method of its development. An extremely important point brought out by Stern requires emphasis; namely, that in spite of many attempts to demonstrate spirochetes in the spinal cord or posterior root lesions of tabes, only four observers have been successful (Noguchi, Versé, Jahnel and Richter). Another feature of the tabetic disease is its association with primary optic atrophy. This has been attributed to the presence of inflammatory lesions at the base of the brain in direct contact with the unmyelinated fibers in the periphery of the optic nerve. However, basilar meningitis is not a universal finding in tabes; in fact, Richter [6] thinks that if present to considerable extent it indicates a coexistent paretic process. Moreover, Spielmeyer's work [7] on the pathology of tabes emphasizes the point that inflammatory and degenerative changes do not parallel each other. Hence the toxic theory of the pathogenesis of tabes was strongly favored by many investigators. It is a view commonly held that the process in tabes is not dependent on the direct action of spirochetes within the nervous system but that the nervous system is damaged by a hypothetical toxin, dependent on the presence of organisms outside the central nervous system as well as within it.

The pathological anatomy of primary optic atrophy has been studied in some detail. The older view, held up to the end of the nineteenth century by Gowers, Erb, Charcot and others, that it depended on a primary degeneration of neurons, either in the retina or in the nerve, is no longer tenable. Léri's observation [8] of numerous intact ganglion cells in complete optic atrophy, and the constant presence of ganglion cells independent of the presence or absence of myelinated fibers in the optic nerve endings, showed clearly that tabetic optic atrophy is not a primary degeneration of the cells of origin of the optic nerve. He considered the process primarily as a periarteritis and endarteritis, with subsequent degeneration of nervous tissue, producing an interstitial neuritis. Stargardt,[9] whose work in 1913 initiated the modern study of the pathological anatomy of primary optic atrophy, demonstrated that the primary changes commence in the intracranial portion of the nerve including the chiasm. He examined the visual apparatus of 19 patients with paresis, 4 with tabo-

paresis, and 3 with tabes. Six of these showed advanced optic atrophy, and 4 others had partial atrophy. The pathological process began in the pial and subpial zones of the nerve, consisting of an undue proliferation of glia fibers, with isolated plasma cells in the overlying pia. The myelin sheaths first became involved, and then the axis cylinders. The intraneural connective tissue and blood vessels showed no early alterations, and could be apparently normal even in far advanced atrophy. Stargardt's findings did not differ materially in paretics and tabetics. Richter [6] agreed with Stargardt and concluded that "the simple optic atrophy in tabes and in paresis is the result of a chronic inflammation which commences in the neighborhood of the chiasm, the tract and the intracranial portion of the optic nerve, and makes its way along the lymph sheaths of the vessels into these tissues."

The next important study was made in 1926 by Behr [10] who examined serial sections from the eye to the geniculate bodies in 9 cases of optic atrophy. He concluded that the degeneration always begins at the nerve margins, in the nerve bundles underlying the pia, and that it always occurs distal to the chiasm. The intracranial portion of the optic nerve is, according to Behr, that part of the visual pathway which is in most intimate contact with the pia and the mesodermal connective tissue septums. He agreed with the older view that exudative changes in these mesodermal tissues are constantly present in tabetic optic atrophy. The principal change in syphilitic primary optic atrophy, therefore, is an inflammatory process in the connective tissue septums of the intracranial portion of the nerve and of the blood vessels which run within them. The primary change is not only in the marginal but also in the parenchymatous glia fibers, and the secondary result is a disturbance of nutrition of the nerve fibers, ending in degeneration. Behr also believed that a toxin of some sort must be partly responsible for optic atrophy, but that its effect was not on the nerve fibers but on the glia fiber system.

The discovery in 1928 by Igersheimer [11] of spirochetes in the sheaths of the optic nerve in a large proportion of cases of syphilitic optic atrophy gave a severe blow to the toxic theory, and since then there has been very general agreement that the degeneration in the fibers of the optic nerve is secondary to syphilitic inflammation of the pia mater surrounding it. There

are still some, however, who believe that optic atrophy, as well as atrophy of the posterior columns in tabes, may, to some extent at least, be due to a toxic or anaphylactic process with selective action on these nerve fibers. This is a very important consideration, not only from the standpoint of the pathologist but also from that of the practical therapeutist. There are today well known therapeutic agents which are thoroughly adequate to combat the inflammatory elements in syphilitic disease of the nervous system, and it is well established that about one-half of the cases of early optic atrophy may be arrested by the prolonged use of intradural treatment and, according to some authors, by artificial fever therapy. If the disease is the result of the direct action of a toxin upon the nervous tissue, one would hardly expect any degree of success in treatment with antisyphilitic remedies which are designed to destroy organisms within the diseased tissue.

The most common defect in syphilitic optic atrophy is concentric constriction of the visual fields. Alterations appear in the fields before any change is apparent in the discs, or even before visual acuity is reduced. Hemianopias, binasal or bitemporal, are considered to be rare in tabetic atrophy, although they have been described by several authors (Stargardt, Fuchs, Kyrieleis, de Schweinitz). As pointed out by Wilbrand and Saenger,[12] these may be pseudohemianopias due to symmetrical defects in the peripheral fields. De Schweinitz [13] emphasized the close similarity between the field defects of pituitary disorders and those of tabes. He quoted Fuchs as entertaining the idea that bitemporal hemianopia is an essential manifestation of the tabetic process, where the lesion attacks the inferior chiasmal fibers. Kyrieleis [14] mentions instances of involvement of the tract with resultant homonymous hemianopia. Thus the distribution of the atrophy in different cases must not be overlooked in considering the problem of the pathogenesis of syphilitic optic atrophy. Moreover, detailed anatomical studies are also helpful in attempting to account for the clinical manifestations of the disease. The following case report illustrates this point and also demonstrates the rôle of chronic inflammation in syphilitic optic atrophy.* [15]

* Work done in the pathological laboratory of the National Hospital, Queen Square, London.

Report of Case

Clinical History: A 57 year old male patient, with a history of loss of sight of the right eye of 20 years duration and of progressive deterioration of vision in the left eye over a period of 3 months, presented a temporal hemianopia with sparing of at least some part of the macula and great constriction of the nasal half of the left visual field. The right eye was completely blind and with the left eye he could only see fingers held directly in front of him.

Ophthalmoscopic examination showed optic atrophy more marked on the right. The left pupil gave the typical Argyll Robertson reaction and the right pupil was not only fixed to light but gave a very feeble contraction on convergence.

There was some evidence of ataxia, more definitely in the legs, and shooting pains of the tabetic type had been present for 7 years. The deep reflexes were reduced in the right arm, and the biceps and triceps jerks were absent on the left, but the knee and ankle jerks were present. There was considerable impairment of pain sensibility all over the body except for small areas on the feet, the hands and the root of the neck.

Examination of the cerebrospinal fluid gave rather mildly positive results, consistent with a predominantly meningeal type of neurosyphilis.

Pathological examination showed that the process was, in fact, that of chronic syphilitic meningitis rather than that of general paresis or tabes dorsalis. There was diffuse thickening of the leptomeninges which were adherent to the dura mater over the dorsal surface of the spinal cord. In addition there was slight general atrophy of the brain but no alteration in the arteries. The posterior roots in the lumbar regions of the cord were rather gray, but there was no evidence of degeneration in the posterior columns either macroscopically or microscopically. In addition to the widespread thickening of the pia-arachnoid, microscopic examination revealed infiltration of these membranes with lymphocytes. The infiltration was fairly intense around the base of the brain, and in some places medium sized vessels near the surface were surrounded with a cuff of lymphocytes. A fairly solid layer of proliferated neuroglial tissue overlay the ependyma covering the floor of the ventricle, and similar layers of proliferated neuroglia had burst through the pia mater covering the ventral surface of the medulla and pons in some places. No loss of nerve cells and no excess of neuroglia or rod cells could be seen in the cerebral cortex, and although an occasional vessel was surrounded by a cuff of infiltration, most of the vessels were intact. The basal ganglia were normal except for infiltration of some of the veins

underlying the ventricular walls. In the spinal cord there was no evidence of tract degeneration nor were the dorsal roots appreciably demyelinated. There was perhaps a slight reduction in the number of fibers passing into and across the substantia gelatinosa of the dorsal horns, but no other evidence of tabes could be seen.

Detailed study of the optic pathways was made in this case. Both optic nerves were very adherent to the dural sheath, so that the latter could not be dissected off. The nerves, chiasm and tracts were extremely thin and grayish on cross section. The intracranial portions of these structures were studied in serial sections in the coronal plane by the Weigert-Pal method. The optic nerves in their orbital portion were also examined at various levels by this method as well as by the Gros-Bielschowsky technic for axis cylinders and by various other stains. The posterior parts of the retina of both eyes were also examined.

The meningeal infiltration seen elsewhere in the nervous system was considerable throughout the intracranial portions of the optic nerves, the chiasm and tracts, but in front of the orbital foramen it was slight and practically none was seen round the most anterior parts of the optic nerves (Fig. 1). In the chiasm the entering vessels were all surrounded by a cuff of lymphocytes, but in front of and behind this level, very few lymphocytes could be seen within the nervous parenchyma except in a narrow zone under the pia mater. Anterior to the optic foramen there was obliteration of the subarachnoid space, the pia-arachnoid being concentrically thickened and adherent also in many places to the dural sheath (Fig. 2). The connective tissue septums were considerably thickened near the surface of the nerve but those near the center were less affected (Fig. 3). The pia-arachnoid covering the intracranial portions of the optic nerves and tracts was also thicker than normal. There was no evident disease of the walls of the small vessels in relation to the optic pathways except infiltration with lymphocytes.

Demyelination was almost complete in the right optic nerve (Fig. 4). In spite of this there were a number of myelin sheaths persisting in the most anterior portion of the nerve, but farther back they disappeared completely. Near the nerve head they were associated with a much larger number of axis cylinders,

which were scattered fairly evenly throughout the core of the nerve but were scanty near its surface. Most of these also disappeared on nearing the optic foramen.

In the left optic nerve the demyelination was also everywhere greatest on the surface, but in the most anterior half inch a sector on the lower and inner aspect was also pale, making the area where myelin sheaths remained roughly crescentic in shape (Fig. 5). This pale sector became less obvious near the optic foramen, the central core of the nerve here becoming fairly evenly myelinated. There was, however, a considerable poverty of myelin sheaths, even in the core of the nerve. In this nerve also axis cylinders were more numerous than myelin sheaths and were found in areas which had undergone complete demyelination (Fig. 6).

Behind the chiasm the right tract contained at all levels much fewer myelinated fibers than the left, but the contrast became greater as one passed backward (Fig. 7). In the right tract the (crossed) myelinated fibers lay near the inferior and outer part of the tract, *i.e.* under the meningeal surface, leaving the greater part of the tract on the side toward the crus cerebri pale. They were, however, separated from the meningeal surface at all levels by a zone of relative pallor, which became wider on passing backward so that near the external geniculate body they all lay fairly deeply and a few could be seen close to the fibers of the crus.

In the left tract (uncrossed) myelinated fibers lay against the side of the midbrain, being everywhere separated from the meningeal surface by a wide zone. They formed a fairly compact bundle, which became less dense on its inferior and outer aspect. They showed very little evidence of degeneration and there was no appreciable loss of fibers on their transit from the optic chiasm to the geniculate body.

Both retinae showed great loss of nerve cells and fibers in the inner layer. In the right eye very few nerve cells remained, but in the left a considerable number were preserved in the neighborhood of the macula, although they were spaced well apart from one another. The granular layers and the rods and cones were everywhere well preserved, and there was only a moderate excess of neuroglial fibers in the layer of nerve fibers with slight thickening of the fibers which normally pass down from this layer to the

inner granular layer. The optic nerves, on the other hand, showed an intense neuroglial sclerosis throughout their intra-orbital course.

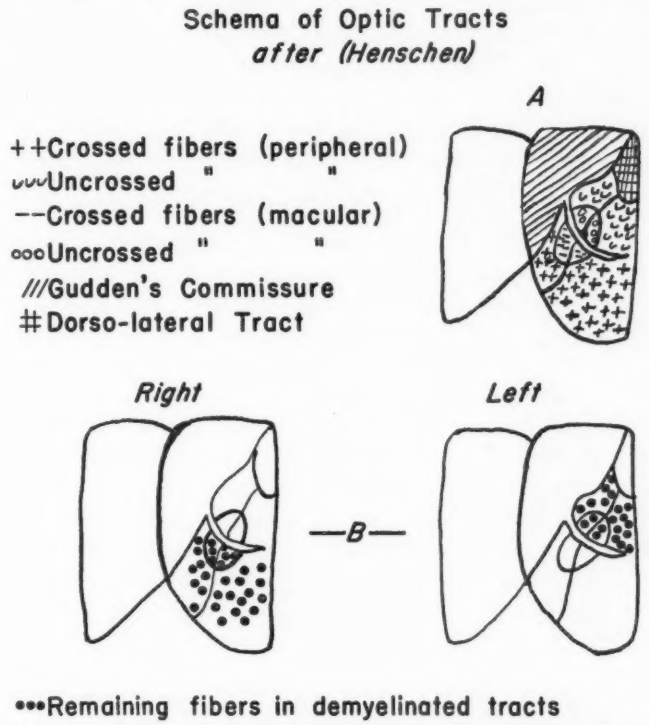
Discussion

From the foregoing description it is seen that lesions were present in the optic nerves, chiasm and tracts, and that the process was primarily inflammatory. These observations point to chronic meningeal inflammation as the cause of the optic atrophy. Stargardt's original observations were fully corroborated: the superficial fibers in the optic nerve were first affected; the myelin was more affected than the axis cylinders; and the inflammatory changes in the meninges were much more evident in the intracranial than in the intra-orbital portion of the optic pathways. One cannot of course draw any hard and fast conclusions on the basis of but one case study. But there can be no doubt that the presence of a chronic syphilitic meningitis in this case had a direct relation to the degenerative process in the visual pathways. This case was uncomplicated by dementia paralytica and showed but little evidence of tabes. Since the optic pathways are an integral part of the central nervous system, the pathology may be identical with that which occurs in other tracts in the nervous system. Thus what is observed pathologically in other syphilitic lesions in the brain may be expected to occur in cases of syphilitic optic atrophy.

In this connection reference should be made to the work of Merritt, Putnam and Campbell,[16] who presented evidence of the rôle of obstruction of blood vessels in the production of the cortical atrophy of dementia paralytica. They suggested that the degenerative changes in the cortex may be secondary to anoxemia produced by endarteritis of small vessels. The histological pictures of lesions in this case of syphilitic optic atrophy bear some resemblance to those produced by experimental obstruction of capillaries of the cortex in dogs. However, it is obvious that many more cases of optic atrophy must be studied pathologically in order to be convinced of the uniformity of the lesions. Most important of all is the study of cases with beginning optic atrophy. These are very difficult to obtain since such cases rarely come to autopsy. The evidence supplied by the study of cases complicated by dementia paralytica contained in the literature

cannot be taken as having a specific bearing on the pathogenesis of the disease.

From the anatomical standpoint this case affords an adequate explanation of the occurrence of bitemporal hemianopia in syphi-



TEXT-FIG. 1. A = anatomical relations of nerve fibers in the optic tract. B = location of the remaining fibers in a case of optic atrophy, shown by the black dots, representing crossed fibers in the right tract and uncrossed fibers in the left.

litic optic atrophy. The position taken up in the tract by the crossed and uncrossed fibers is in agreement with Henschen's diagrammatic representation of the position of fibers from different parts of the retina. It is probable that the separation of the fibers of the papillomacular bundle from those coming from the more peripheral parts of the field is not so definite as this diagram would suggest. Brouwer,[17] in his experiments on monkeys, found

that the papillomacular fibers were widespread both in the nerve and tract, and mingled with those from the peripheral parts of the retina. There seems, however, to be no doubt that the position of the crossed and uncrossed fibers in the optic tracts is different in man from that which he found in the monkey, where the crossed fibers take up a position in the inferior and inner part of the tract. In Henschen's diagram and in the case reported here they lie on its inferior and outer surface, the uncrossed fibers being throughout more deeply placed (Text-Fig. 1). This position of the crossed fibers, in close relation to the meningeal surface of the optic tract, makes them more likely to be affected by inflammation of the meninges. In the case reported here they appeared to recede from the surface on passing backward, becoming less numerous and showing more evidence of degeneration. The zone of pallor on the meningeal surface of the right optic tract was thus in every way similar to the pale superficial zone in the left optic nerve, and suggested that the progressive degeneration in this tract was dependent on a similar pathological process, which is presumed to be the passage of toxic products of inflammation inward from the meninges. This degeneration of the meningeal surfaces of the optic tracts, by affecting only the (crossed) fibers from the nasal half of the retina, may thus be a factor in producing the disproportionate reduction in the temporal halves of the fields, which is not so very uncommonly seen in syphilitic optic atrophy. It is not denied that involvement of fibers as they cross in the chiasm may also contribute to this partial hemianopia. Such an involvement was evident in this case, but it appeared insufficient to account for the marked loss of vision in the temporal field as a considerable proportion of fibers crossed to the right optic tract. It is probable that normally there are more crossed than uncrossed fibers, whereas in the case reported here there were fewer. But the progressive diminution in the number of myelinated fibers in the right tract, and the way in which they receded from the meningeal surface, indicated an attack by the syphilitic process in the tract as well as in the chiasm.

Summary and Conclusions

A preliminary study of the pathogenesis of syphilitic optic atrophy is presented. It is suggested that there is good evidence

of a fairly close relationship between the inflammatory and degenerative reactions in the nervous system. Not only the pathological findings but also the response to treatment of some cases of syphilitic optic atrophy makes such a hypothesis tenable. Nevertheless, detailed studies of more cases must be made. A rather precise clinical and anatomical correlation is presented between the ophthalmoscopic manifestations of this disease and the distribution of the pathological lesions in the optic pathways. The position of the (crossed) nasal fibers on the meningeal surface of the optic tract makes them liable to be affected by inflammation and this may explain the bitemporal hemianopia found in some cases of syphilitic optic atrophy.

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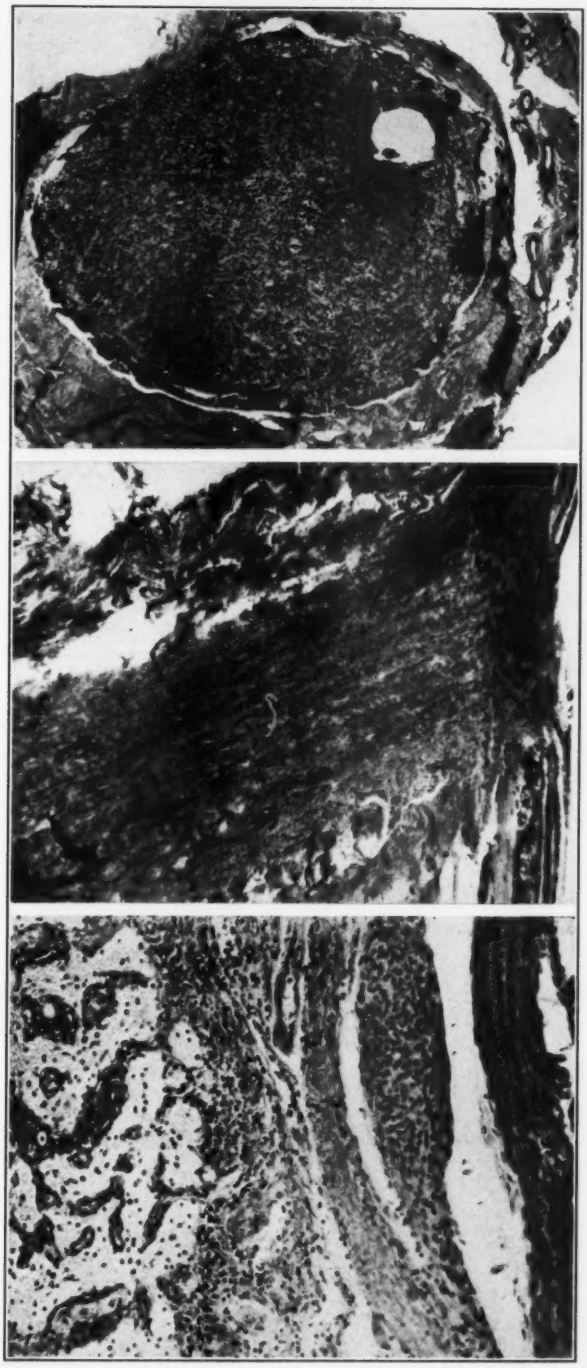
DESCRIPTION OF PLATES

PLATE 45

FIG. 1. Edge of the intracranial portion of the left optic nerve showing meningeal infiltration. Van Gieson's stain.

FIG. 2. Longitudinal section of the right optic nerve, near the retina, showing obliteration of the subarachnoid space and thickening of connective tissue. Van Gieson's stain.

FIG. 3. Right optic nerve showing thickening of the pia-arachnoid and connective tissue septums. Van Gieson's stain.



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2

1

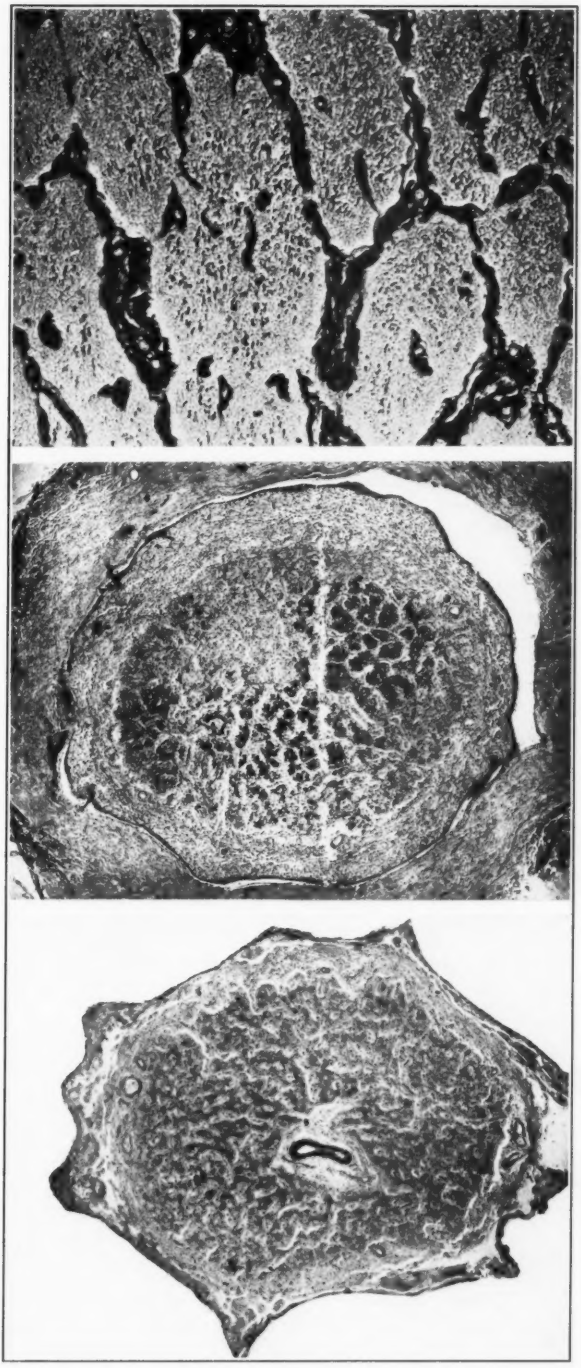
Epstein Syphilitic Optic Atrophy

PLATE 46

FIG. 4. Right optic nerve with almost complete demyelination. Weigert-Pal method.

FIG. 5. Left optic nerve with preservation of myelin sheaths in crescentic fashion on the upper and outer aspect. Weigert-Pal method.

FIG. 6. Left optic nerve showing the presence of numerous axis cylinders. Gros-Bielschowsky method.



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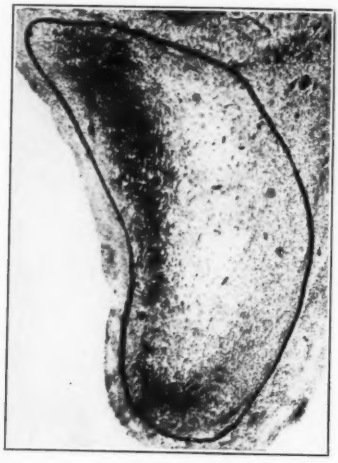
4

Epstein Syphilitic Optic Atrophy

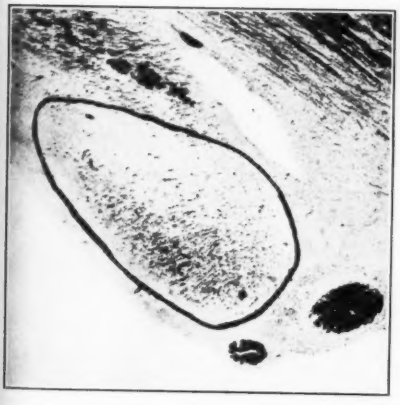
PLATE 47

FIG. 7. Optic tracts. A = 0.5 cm. behind chiasm; B = 1 cm. behind chiasm; C = 1.5 cm. behind chiasm.

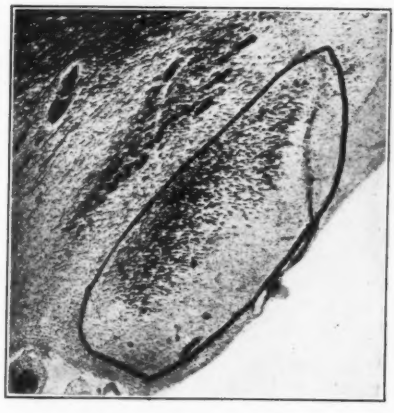
The fibers remaining in the right optic tract, coming from the nasal half of the left retina, lie ventrolaterally on the exposed surface of the tract. Those in the left tract, from the temporal half of the retina, lie dorsomesially, deep in the tract. Weigert-Pal and carmine stain.



A



B



C

Right

Left

Epstein

Syphilitic Optic Atrophy

THE TRANSMISSION OF LYMPHOGRANULOMA VENEREUM TO THE GUINEA PIG *

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Many investigators have attempted to transmit lymphogranuloma venereum to the guinea pig, using a variety of routes of inoculation. It will be convenient to review the results obtained by these workers under the headings of the routes employed.

Subcutaneous Inoculation

The earliest experiments consisted of inoculating human lymphogranulomatous pus or gland material subcutaneously in the groin. Durand, Nicolas and Favre (1913),[1] Phylactos (1922),[2] and Frei and Hoffman (1927)[3] obtained only negative results. Ravaut and Sheikevitch (1921),[4] and Ravaut, Boulin and Rabeau (1924)[5] reported the development of a transitory swelling in the inguinal region. Gamna (1923)[6] produced an inguinal adenitis which disappeared spontaneously and which histologically resembled human lymphogranulomatous glands. He was also able to transmit the infection for 3 passages in guinea pigs.[7] De Bella (1924),[8] Virgillo (1925),[9] Gay Prieto (1927),[10] Freund and Reiss (1931),[11] Nicolau (1932),[12] Findlay (1933), [13] and Löhe and Schlossberger (1935)[14] also reported the production of enlarged inguinal glands.

De Blasio (1931)[15] injected human pus directly into exposed cervical nodes and produced a local adenitis as well as a generalized lymphadenopathy with enlargement of the spleen and sometimes of the liver. Unsuccessful attempts were made to pass the disease by gland to gland inoculation.

The first definitely proved transmission of the disease to the guinea pig was accomplished by Meyer, Rosenfeld and Anders (1931)[16] who showed that antigens prepared from infected animal tissues gave positive Frei reactions in lymphogranulomatous

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individuals. From 9 of 10 cases of lymphogranuloma venereum 19 of 22 guinea pigs were infected. The best results were obtained by bruising the inguinal region prior to inoculation.

Ravaut, Levaditi, Lambling and Cachera (1932)[17] implanted human lymphogranulomatous rectal tissue subcutaneously in guinea pigs and obtained enlargement of the regional glands. An emulsion of the glands produced a meningo-encephalitis in monkeys. This experiment was corroborated by Laederich, Levaditi, Mamou and Beauchesne (1932).[18]

Wassén (1935),[19] Löhe, Rosenfeld, Schlossberger and Krumeich (1933),[20] and Hashimoto (1936) [21] reported infection of guinea pigs by the use of lymphogranulomatous animal tissue.

Intracerebral Inoculation

Freund and Reiss (1931)[11] claimed to have transmitted the disease to guinea pigs by this route using tissue from a rabbit which they regarded as lymphogranulomatous. Levaditi, Ravaut, Lépine and Schoen (1932),[22] and Caminopetros, Phylactos and Photakis (1932),[23] however, reported negative results following intracerebral inoculation of virus. Findlay (1933),[13] using both human and mouse lymphogranulomatous tissue, infected 2 of 23 guinea pigs and caused a meningo-encephalitis in mice with emulsions of their brains. Wassén (1935)[19] reported that he and Hellerström were unable to produce any lesions following injection of monkey or mouse brain material. Toyama, Hasegawa and Ichikawa (1936)[24] found that intracerebral inoculation of human material occasionally produced definite signs of meningo-encephalitis in guinea pigs; at other times the infection was symptomless.

Intradermal Inoculation

Infection of guinea pigs by this route was first accomplished by Wassén (1934)[25] who employed an inoculum of lymphogranulomatous mouse brain. A papule arose at the site of inoculation with involvement of regional lymph glands. Bizzozero and Midana (1935)[26] reported the production of cutaneous lesions by the use of human pus; the satellite glands, however, were unaffected. Tasaki (1937)[27] obtained a papule with regional adenopathy following inoculation of virulent mouse brain emulsion.

Other Routes of Inoculation

Wassén (1935)[19] did not observe any effects following inoculation of lymphogranulomatous monkey brain into the peritoneal cavity of guinea pigs. Caminopetros (1934)[28] reported infection of guinea pigs by intrapulmonary inoculation of an emulsion of human lymphogranulomatous glands.

The results just reviewed would indicate that the transmission of the virus of lymphogranuloma venereum to guinea pigs, especially by the subcutaneous route, can be readily accomplished. Proof, however, is lacking in many of the reports quoted above that the pathological changes in the inoculated guinea pigs were due to the virus of lymphogranuloma venereum and not to the lighting up of a latent *Pasteurella pseudotuberculosis rodentium* infection, the pathological picture of which may be confused with that of lymphogranuloma venereum. In addition, in instances in which the virus was definitely transmitted, it was not found possible to infect regularly all of the inoculated animals. Thus, Findlay (1933)[13] using 7 different samples of human lymphogranulomatous material for subcutaneous inoculation definitely infected only 0 to 44 per cent of the guinea pigs. Similarly, D'Aunoy, von Haam and Lichtenstein (1935)[29] reported that only 50 per cent of the guinea pigs injected with their mouse brain virus subcutaneously developed buboes. Wassén [19] reviewing the field said: "The guinea pig does not seem to be an animal very receptive of the virus of lymphogranuloma inguinale."

It was the purpose of this investigation, which was carried out in 1935, to discover with what regularity the guinea pig could be infected with the authors' strain of the virus of lymphogranuloma venereum. Results are recorded of the inoculation of guinea pigs by many routes using a strain of the virus which at the time, in a concentration of 10^{-3}, was capable of causing a fatal meningoencephalitis in mice within 7 days. In order to determine whether the pathological changes induced in the guinea pigs were lymphogranulomatous, it was necessary to demonstrate the presence of the virus in the morbid tissue. Two methods were employed for that purpose: (*a*) the transmission of the disease to mice by intracerebral inoculation of the tissue, and (*b*) the preparation of potent and specific Frei antigen from the tissue.

Materials and Methods

Virus was introduced by as many routes as possible in order to determine the most satisfactory for the induction of disease. The subcutaneous, intradermal and genital routes were employed in an attempt to reproduce a clinical picture similar to that occurring in the inguinal variety of the human disease. Concomitant inoculation into the anterior chamber of the eye and into the brain was employed as a result of the work of Phylactos (1935)[30] who claimed to have produced in this manner rapid development of lymphogranulomatous inflammation of the central nervous system of the rabbit. Inoculation into the rectal mucosa was chosen in view of the accumulation of evidence by Grace and Henry[31] that anorectal lymphogranuloma venereum is often acquired by the direct deposition of the virus on the anal and rectal mucosae.

White or cream colored guinea pigs weighing from 250 to 275 gm. obtained from one breeder were used. Such an animal allows changes in the skin to be seen readily and can be easily handled. The virus used throughout this work was an emulsion in sterile physiological saline of mouse brain infected with the strain isolated by the authors in 1934.[32] A 10 per cent suspension was employed except where otherwise indicated. Normal mouse brain prepared in the same manner as the infective material or inactivated virus emulsions were used as control inoculums. Inactivation was accomplished by heating at 60° C. for 2 hours on one day and for 1 hour on the next. All inoculums, whether of mouse or of guinea pig origin, were tested for bacteriological sterility by aerobic and anaerobic culture before use. Animals were examined daily for at least 1 month after inoculation and changes in their clinical condition were carefully noted.

Histological examination was made of tissues embedded in paraffin and stained with hematoxylin and eosin.

A. Routes of Inoculation

1. Subcutaneous Route: Inoculations were made into the shaved right groin, the uninoculated left groin serving as a control. 0.75 cc. of a suspension of virus of varying concentrations (1:5, 1:10, 1:20, 1:100, and 1:500) were used to determine the lowest which would produce in all the guinea pigs a readily measurable, large

inguinal mass. The 1:10 concentration was found to be most suitable and was employed in 40 animals. One died and 14 were sacrificed at approximately 2 day intervals between the 3rd and 21st days after inoculation. The enlarged inguinal glands were removed from the sacrificed animals for histological study, for the preparation of Frei antigen, and for injection into mice or other guinea pigs. Histological examination was also made of the spleen, aortic and lumbar glands, and left inguinal glands. The remaining 25 animals were observed until the gland masses had disappeared completely.

A 1:10 concentration of normal mouse brain was injected into 5 control animals. One was sacrificed on the 5th day and the inguinal glands excised for section.

2. *Intradermal Route:* 0.1 to 0.15 cc. of virus suspension was introduced into the shaved skin of the right groin or abdominal wall of 12 guinea pigs. Six of these animals received heat-killed virus concomitantly in the skin of the left side. The lesions at the sites of inoculation as well as the inguinal glands were taken from 5 sacrificed animals for histological study and for mouse inoculation.

3. *Vaginal Route:* 0.1 cc. of virus suspension was injected into the vaginal mucosa of 3 animals. Two controls received inactivated virus. A small straight artery forceps was used as a speculum to facilitate the procedure. One of the infected animals was sacrificed on the 6th day and the vagina, uterus, rectum and inguinal glands were dissected out for microscopic examination.

4. *Intrapreputial Route:* Inoculation of 0.1 cc. of virus suspension was made into the prepuce of 3 guinea pigs. One was sacrificed on the 6th day and histological examination was made of the penis and inguinal glands. Two control animals were given a suspension of killed virus.

5. *Intratesticular Route:* 0.1 cc. of virus suspension was injected into the left testis of 4 animals. Three others received a similar volume of virus inactivated by heat. On the 3rd day the scrotal contents of 2 test and 2 control animals were taken for section.

6. *Rectal Route:* 0.5 cc. of virus suspension was introduced into the rectal mucosa of 3 guinea pigs. Fifteen days later Frei tests were performed on the animals with mouse brain antigen.

7. Intraperitoneal Route: Three animals received 0.5 cc. of virus suspension intraperitoneally.

8. Intracerebral Route: Seven animals were used in this experiment. Through a trephine opening in the skull virus was inoculated into the underlying parietal lobe of the brain. One animal received 0.2 cc., 5 animals 0.3 cc., and 1 animal 0.5 cc. of infectious material. Two died and their brains were removed for histological study, for the preparation of Frei antigen, and for mouse inoculation.

9. Corneal Scarification: 0.03 cc. of virus suspension was deposited on the scarified cornea of 2 guinea pigs. The inoculated eye of 1 animal was excised for further study on the 14th, and of the other, on the 19th day. Two control animals were given an emulsion of heat-killed virus.

10. Inoculation into Anterior Chamber of Eye: Inoculation of virus suspension into the anterior chamber was carried out in 5 guinea pigs; 4 received 0.03 cc., and 1, 0.01 cc. Two control animals were injected with 0.03 cc. of inactivated virus. Animals were sacrificed on the 5th, 10th and 20th days and the inoculated eyes removed for histological examination.

11. Concomitant Inoculation into Anterior Chamber of Eye and into Brain: This group consisted of 4 animals which were injected with 0.03 cc. of virus suspension into the anterior chamber of the eye and concomitantly with 0.2 cc. of the same emulsion intracerebrally. One died on the 2nd day. The brain was removed for section and for preparation of Frei antigen; the inoculated eye was also taken for microscopic examination.

B. Passage of Lymphogranulomatous Guinea Pig Material to other Guinea Pigs

Guinea pigs which received mouse brain virus suspension subcutaneously in the groin were sacrificed on the 5th, 6th and 13th days after inoculation and the inguinal masses dissected out aseptically. Directly after excision each material was emulsified in approximately 4 times its volume of sterile physiological saline and then inoculated by the subcutaneous route into a new group of guinea pigs. Four of the latter animals were sacrificed and the enlarged inguinal glands used for section and for further guinea

pig passage. Transmission was not attempted for more than 2 successive passages.

C. Preparation of Frei Antigen from Lymphogranulomatous Guinea Pig Material

Frei antigens were made from material obtained from 7 of the 40 animals described under "subcutaneous route." For this purpose the enlarged inguinal glands of 5 guinea pigs sacrificed on the 3rd, 6th, 8th, 9th and 15th days respectively were employed. A 20 per cent suspension of the morbid tissue was prepared by trituration with sterile physiological saline and was heated in the usual manner. In addition, portions of the emulsions of the 5th day glands which were used in the 1st guinea pig to guinea pig passage (*vide supra*) were heated to make antigens. All of the antigens were bacteriologically sterile before and after heating and were tested for potency and specificity on 3 normal and 6 lymphogranulomatous individuals. Reading of reactions was made by the measurement of the diameter of the papule produced 72 hours after the performance of the test. Emulsion of normal guinea pig inguinal gland prepared in the same manner as the lymphogranulomatous material was used as control.

D. Intracerebral Inoculation of Lymphogranulomatous Guinea Pig Material into Mice

Inguinal masses removed from guinea pigs on the 6th and 12th days after subcutaneous inoculation in the groin with virulent mouse brain were emulsified in 4 times their volume of sterile physiological saline. 0.03 cc. of the emulsion was inoculated intracerebrally into mice.

The primary lesion and the enlarged inguinal glands resulting from the intradermal inoculation of virus were excised and dealt with in a like fashion. The injected mouse brains were examined histologically.

E. Frei Tests of Lymphogranulomatous Guinea Pigs

Frei tests were performed on 18 guinea pigs, 15 of which had received virus by the subcutaneous, and 3 by the rectal route. The latter group was tested 15 days after inoculation. Of the

former group 5 were tested at 7 and again at 35 days, 5 at 44 days, and 5 at 65 days after injection.

Tests were carried out by the introduction of 0.1 cc. of mouse brain Frei antigen into the shaved skin of the right half of the abdominal wall. Control tests with heated emulsion of normal mouse brain were performed on the left half of the abdominal wall. Reading of reactions was made daily for 5 to 7 days after testing.

Results

A. Inoculation of Guinea Pigs with Virus by Various Routes

1. Subcutaneous Route:

(*a*) *Using a 1:10 Concentration of Virus:* Enlargement of the inguinal glands on the inoculated side was evident in each of the 40 animals on the 2nd day and increased until a maximum size was reached on the 8th to the 11th day. Gradual recession then occurred until by the end of 1 month the inguinal swellings disappeared. In a small number of animals a fibrous knot 0.5 to 0.8 cm. in diameter persisted after the inguinal mass had subsided. The glands on the uninoculated side were unaffected.

Infection was manifest as hot, hard, non-fluctuant, nodular masses adherent to the shiny overlying skin which had a purple-red tint. In very extensive involvement the masses became attached to the subjacent muscle of the abdomen and thigh, frequently causing retraction of the hind limb. Infected animals generally did not appear ill, although several lost weight during the acute stage; only 1 died.

Daily measurements of the size of the masses were made in three dimensions, *i.e.* anteroposterior, from flank to midline, and dorsoventrally. These measurements were denoted as length, breadth and thickness respectively. Table I shows the size of the masses at intervals after inoculation. The figures shown in the second column are a true average of 4 groups of 5 animals each, 1 group of 7 animals, and 1 group of 13 animals. Each group received material of a different passage of virus. The maximum and minimum sizes recorded are those of the largest and smallest masses respectively encountered among all of the animals on any particular day after inoculation.

(*b*) *Using other Concentrations of Virus:* Inguinal swellings were observed in all of the 13 animals inoculated with emulsions of concentrations 1:5, 1:20, and 1:100. Only 1 of the 3 guinea pigs, however, developed an adenitis with a 1:500 virus suspension. The size of the masses in the animals injected with a 1:5 virus suspension was approximately the same as that obtained with a 1:10 suspension. Emulsions of 1:20 and 1:100 produced masses which were smaller and subsided sooner than those produced with a 1:10 concentration. The masses evoked by the 1:20 suspension, however, were slightly larger than those following inoculation with the more dilute material.

TABLE I

Size of Inguinal Masses of 40 Guinea Pigs Following Subcutaneous Inoculation with 0.75 cc. of 10% Virus Suspension

Day after inoculation	True average size	Maximum size	Minimum size
	cm.	*cm.*	*cm.*
2nd	2.1 x 1.9 x 0.8 *	3.0 x 2.5 x 1.0	1.5 x 1.5 x 0.8
4th	2.2 x 2.0 x 0.9	3.0 x 2.5 x 1.0	1.8 x 1.5 x 0.8
6th	2.5 x 2.3 x 1.2	3.5 x 3.5 x 1.5	1.5 x 2.0 x 0.5
8th	2.6 x 2.2 x 1.1	3.5 x 2.5 x 1.5	1.5 x 2.0 x 0.8
10th	2.6 x 2.1 x 1.0	4.0 x 3.0 x 2.0	1.5 x 1.5 x 0.8
13th	2.1 x 1.7 x 0.9	3.0 x 2.5 x 1.5	1.5 x 1.0 x 0.5
15th	1.8 x 1.3 x 0.8	3.0 x 1.5 x 1.0	1.0 x 0.5 x 0.5
19th	1.3 x 1.1 x 0.5	2.0 x 1.5 x 0.5	0.5 x 0.5 x 0.5
27th	0.6 x 0.6 x 0.5	0.8 x 0.8 x 0.5	0.5 x 0.5 x 0.5

* The measurements are expressed as length x breadth x thickness.

(*c*) *Using a 1:10 Concentration of Normal Mouse Brain:* All of the 5 animals showed slight erythema of the inoculated inguinal area in 48 hours. In 4 of the animals the homolateral lymph glands increased slightly in size on the 3rd day, measuring approximately 0.5 by 0.3 by 0.3 cm. and did not become any larger. They were freely movable, were only apparent on palpation, and persisted for from 1 to 5 days. The reaction to normal mouse brain could not be confused with that produced by lymphogranulomatous material.

(*d*) *Pathological Examination:* Grossly the inguinal masses appeared yellowish in color, irregular in outline, and displayed many areas of hemorrhage. They were attached to the overlying skin up to the 15th day after inoculation. The subcutaneous

tissue was usually markedly injected. In those cases in which the masses were adherent to the abdominal or thigh musculature the involved muscle tissue was thickened and hemorrhagic. Hemisection of the mass revealed the lymph glands as one or more firm, grayish nodular areas embedded in thickened, spongy periglandular tissue. The amount of periglandular involvement depended on the stage of the disease.

Histological examination was made of inguinal masses excised 3, 5, 6, 8, 9, 13, 15 and 21 days after inoculation.

The skin showed no alteration in the epidermis or appendages. An infiltrate of fibroblasts, polymorphonuclear leukocytes, lymphocytes and macrophages was present in the deeper layers of the corium during the 3rd to the 9th days. A similar infiltrate was seen between the muscle bundles of the adherent thigh and abdominal musculature and in the subcutaneous fat.

The periglandular tissue was the most severely affected and constituted the greater portion of the inguinal mass. The degree and type of pathological change observed depended on the time elapsing between inoculation and excision. The extent of involvement increased up to the 9th day, after which it slowly decreased. At first an acute inflammatory reaction was noted characterized by edema, hemorrhage, much granulation tissue and areas of dense infiltration with polymorphonuclear leukocytes, macrophages and occasional lymphocytes. After the 9th day these cells began to disappear, especially the polymorphonuclear leukocytes, and beginning organization of granulation tissue became evident. Giant cells of the Langhans' type were seen on the 13th and subsequent days. By the 21st day no periglandular involvement was apparent.

The lymphoid tissue was moderately hyperplastic. Considerable numbers of polymorphonuclear leukocytes were scattered diffusely throughout the gland and were not arranged to form the stellate abscesses encountered in human lymphogranulomatous buboes.

Eosinophilic bodies similar to the Gamna-Favre bodies were present in the infiltrated areas of the periglandular tissue and infrequently in the gland tissue. They were intra- and extracellular, circular or elliptical in shape, homogeneous in appearance, and measured 1 to 4 μ. Structures of similar morphology

and tinctorial affinity were occasionally encountered in uninoculated and apparently uninvolved lymph nodes.

No pathological change was demonstrable in the left uninoculated glands, the aortic or lumbar nodes, the spleen, or other organs.

Sections of the inguinal node of the animal that received normal mouse brain revealed nothing but a small degree of infiltration of the surrounding areolar tissue with a few polymorphonuclear leukocytes and macrophages.

2. Intradermal Route: All of the 12 animals developed within 48 hours at the site of inoculation with virus a well raised erythematous papule measuring 8 to 15 cm. in diameter. Necrosis of the central portion was present in 8 of the animals at this time. Slight increase in the size of the papule occurred until the 4th day, with concurrent increase in the degree of erythema and extent of necrosis. In one instance the papule did not break down until the 9th day. Regression of the lesions began on the 7th to 10th day and was practically complete 2 weeks later. The homolateral inguinal glands became enlarged in 8 animals by the 4th, in 1 by the 9th, in another by the 13th day, and in 2 animals there was no effect. The glands, which persisted for about 3 weeks, were 0.8 to 1.5 cm. in diameter, were firm, discrete, bean-shaped nodules and never reached the size of those caused by subcutaneous inoculation.

Heat-killed virus uniformly produced at the end of 48 hours low non-erythematous to faintly erythematous papules 2 to 3 mm. in diameter. On the 4th day the papules were slightly larger but never exceeded 3 to 4 mm. They disappeared after 5 to 21 days. No enlargement of the regional lymph glands occurred.

The primary lesion following intradermal inoculation of virus showed, histologically, necrosis of the epidermis and papillary layers of the corium, acanthosis, spongiosis and elongation of the rete pegs. In the corium an increase in fibrous tissue elements and an infiltrate of polymorphonuclear leukocytes, macrophages and some lymphocytes were seen; giant cells were observed in the older lesions. The involved lymph glands showed moderate hyperplasia.

Histological examination of the skin reaction to heat-killed virus revealed a normal epidermis and small collections of fibro-

blasts, macrophages and lymphocytes deep in the corium. No changes were apparent in the inguinal lymph glands of the same side.

3. Vaginal Route: Erythema and edema of the vulva and vaginal walls developed 24 hours after introduction of virus into the vaginal mucosa. At 48 hours small ulcers appeared in 2 animals and a scarlet, hard infiltrated area developed in the 3rd animal at the site of inoculation. A mucosanguineous or mucopurulent vaginal discharge was also evident at this time. Bilateral inguinal adenopathy developed by the 4th day, the enlarged glands resembling those produced by intracutaneous inoculation. The genital involvement began to subside after the 4th day and by the 13th day both the local disease and the adenopathy had disappeared completely.

Heat-killed virus produced no demonstrable change in the external genital organs or in the inguinal glands of the 2 control animals.

Histological examination of the vagina showed a thickening of the malpighian layer of the mucosa as well as edema and extensive infiltration of the submucosa with polymorphonuclear leukocytes and macrophages. The rectum and uterus were normal. The regional lymph glands revealed a mild hyperplasia and the presence of the eosinophilic bodies described above.

4. Intrapreputial Route: Erythema, edema and induration of the prepuce and shaft of the penis occurred in the 3 animals 24 hours after injection of virulent material, and retraction of the prepuce was impossible. In 1 animal small crusted lesions appeared on the prepuce on the 4th day. Regression began about the 6th day although some erythema and swelling persisted until the 25th day. The satellite glands became enlarged 4 days after inoculation and receded at about the same time as the penile involvement. Inoculation of heat-killed virus produced no apparent effect.

Sections of the penis showed acanthosis, edema, hemorrhage and dense infiltration of the corium, subcutaneous tissue, tunica albuginea and corpora cavernosa. The infiltrate was composed largely of polymorphonuclear leukocytes, some macrophages and fibroblasts. The regional inguinal glands exhibited a moderate hyperplasia.

5. Intratesticular Route: In all of the 4 animals on the day following injection with virus the scrotal contents on the inoculated side were swollen and the overlying skin was hot, erythematous and edematous. The inflammatory process began to subside 3 days later and by the 6th day no sign of disease was present. The homolateral inguinal glands increased slightly in size. No clinical manifestations of disease appeared on the uninoculated side or in the animals that received heat-inactivated virus.

On removing the infected scrotal contents the epididymis was enlarged and hemorrhagic; the testis, however, was only a little increased in size and only slightly hyperemic.

Histologically the tunica vaginalis immediately surrounding the epididymis showed edema, considerable extravasation of blood, and was heavily infiltrated with polymorphonuclear leukocytes and macrophages. The infiltrate was present also in the supporting tissue of the epididymis and in places invaded the epithelium and lumen of the ducts. The pathological change in the testis was less severe. A similar infiltrate and many dilated blood vessels occupied the interstitial tissue.

The homolateral inguinal lymph glands were hyperplastic.

There was no microscopic evidence of spread of infection from the inoculated to the uninoculated testes or epididymes. No pathological change was detected in the animals that received heat-inactivated virus.

6. Rectal Route: The 3 inoculated animals remained alive and well.

7. Intraperitoneal Route: The 3 inoculated animals remained alive and well.

8. Intracerebral Route: Five of the 7 guinea pigs that received virus intracerebrally remained alive and well. One of the remainder died on the 3rd and the other on the 14th day without having shown any signs of illness. Emulsions of the brains of the dead animals produced neither typical symptoms of lymphogranuloma venereum in white mice nor positive Frei reactions in known lymphogranulomatous individuals. Histological study revealed only cerebral hemorrhage.

9. Corneal Scarification: Neither gross nor microscopic changes resulted from this procedure.

10. Inoculation into Anterior Chamber of Eye and

11. Concomitant Inoculation into Anterior Chamber of Eye and into Brain: Since lesions of the eyes alone developed in both these groups of animals the results are described together. On the day following inoculation all of the 9 guinea pigs displayed hyperemia of the conjunctiva and a milky appearance of the cornea. The hyperemia increased in severity and was most marked at the corneoscleral junction. In 5 animals partial or complete opacity of the cornea was evident by the 3rd to the 10th days. No purulent exudate was present. In 2 animals the eyeball became misshapen and in 2 others the pupil became constricted by the 10th day. The reaction then began to subside and involution was complete by the end of the 3rd week.

Histologically the changes were those of interstitial keratitis and iridocyclitis. The cornea showed some vacuolization and degeneration of the epithelium together with a moderate infiltration of polymorphonuclear leukocytes in the substantia propria. The anterior chamber contained small collections of polymorphonuclear leukocytes and lymphocytes. A dense infiltrate of polymorphonuclear leukocytes and occasional round cells was seen at the corneoscleral junction and adjoining sclera, in the ciliary body, the ciliary processes and the iris. Dilatation of the blood vessels and edema of the tissues at the angle of the anterior chamber were also present. No choroiditis or retinitis was observed. By the 20th day the reaction had subsided and very little change was demonstrable.

Heat-killed virus produced no effect which was detectable on gross or microscopic examination.

B. Passage of Lymphogranulomatous Guinea Pig Material to other Guinea Pigs

The size and the gross or microscopic appearance of the inguinal masses were the same whether obtained by inoculation of virulent mouse brain or of infected guinea pig gland. The largest masses followed the use of inoculum derived from glands removed 5 to 7 days after injection. Guinea pig to guinea pig transmission was carried on for 2 successive passages without apparent loss of virulence of the virus.

C. Reactions Produced in Humans with Frei Antigen Prepared from Lymphogranulomatous Guinea Pig Glands

By the use of the same lymphogranulomatous individual for the performance of the Frei test with different antigens it was found that the size of the reaction was influenced by the length of time elapsing between inoculation and removal of the glands from which antigen was made. Thus, antigens prepared from glands removed 3, 5, 6, 9 and 15 days respectively after inoculation produced on an average papules 7, 8, 9, 13 and 7 mm. in diameter respectively. The largest papules exhibited necrotic centers, and in the same individual were approximately equal in size to the reaction produced by standardized lymphogranulomatous mouse brain antigen.

Control emulsion of normal guinea pig gland evoked a papule 4 mm. in diameter in lymphogranulomatous and non-lymphogranulomatous individuals. Lymphogranulomatous guinea pig gland emulsion yielded a papule 4 mm. in diameter in non-lymphogranulomatous individuals.

D. Intracerebral Inoculation of Lymphogranulomatous Guinea Pig Material into Mice

Mice that received gland material excised on the 6th and 12th days after inoculation, as well as those that received emulsion of primary lesion, developed a meningo-encephalitis typical of lymphogranuloma venereum. Signs of illness appeared within 2 days and progressed in severity until all of the mice were dead between the 6th and 8th days after inoculation.

E. Frei Reactions of Lymphogranulomatous Guinea Pigs

No reaction was elicited which could be interpreted as a positive Frei test. The responses to the test and control antigens could not be differentiated from each other. At the end of 48 to 72 hours all of the 15 animals yielded an erythematous papule 4 to 6 mm. in diameter which was occasionally surrounded by a small areola. By the end of a week the papules disappeared.

Summary and Conclusions

It has been found possible regularly to infect 100 per cent of the guinea pigs inoculated by a variety of routes with the authors'

strain of the virus of lymphogranuloma venereum. Transmission was accomplished by injecting an emulsion of lymphogranulomatous mouse brain (1) subcutaneously in the groin, (2) intradermally, (3) into the vaginal mucosa, (4) intrapreputially, (5) intratesticularly, and (6) into the anterior chamber of the eye. Infection did not result when the virus was introduced intraperitoneally, into the rectal mucosa, intracerebrally or intracorneally. Regardless of the route of inoculation the disease in all instances remained localized and healing occurred spontaneously.

The essential pathological changes were formation of granulation tissue and extensive infiltration with polymorphonuclear leukocytes, macrophages and lymphocytes. Although necrosis was evident in some tissues the frank pus commonly encountered in human lymphogranuloma venereum was not seen.

Conclusive evidence that the pathological change was due to the virus of lymphogranuloma venereum was obtained by demonstrating the virus in the morbid tissue. To this end it was shown that emulsions of such tissue caused a typical lymphogranulomatous meningo-encephalitis in mice and also that these emulsions inactivated by heat yielded positive Frei reactions when tested on lymphogranulomatous individuals.

Heat-inactivated virus or emulsions of normal mouse brain inoculated in the same manner as virus did not elicit any clinical or histological response which could be confused with that due to lymphogranuloma venereum.

Subcutaneous inoculation of virus in the groin induced a disease picture resembling the inguinal type of lymphogranuloma venereum found in man. The inguinal masses produced were readily measurable and larger than those observed by the majority of workers in this field. Generalized infection, as reported by Nicolau,[12] De Blasio,[15] and Meyer, Rosenfeld and Anders [16] did not occur. Successive transmission of the disease from guinea pig to guinea pig was effected by subcutaneous injection of involved gland material.

A reaction similar to the papular or nodular human primary lesion followed intradermal inoculation. The degree of regional lymphatic involvement was smaller than that following subcutaneous injection.

The introduction of virus into the vaginal mucosa and prepuce

evoked a disease process like that found in early local invasion of the external genitalia in man. Due, perhaps, to the relatively large dose of virus administered to the guinea pig, the pathological change occurred not only in the superficial tissues with the production of a primary lesion but also extended into the deeper layers. Satellite adenopathy was present.

Intratesticular injection of virus caused an epididymitis and orchitis accompanied by hyperplasia of the homolateral lymph glands.

An interstitial keratitis and iridocyclitis was brought about by the introduction of virus into the anterior chamber of the eye.

Guinea pigs with active or healed lymphogranulomatous lesions, injected intradermally with Frei antigen, gave reactions which approximated those elicited by control antigen. Whether the non-specific response to the mouse brain protein masked the specific reaction to inactivated virus was uncertain. One would expect, however, a true positive reaction to have been larger and more acutely inflammatory in appearance than any of those observed in this work. For the present, therefore, the reactions cannot be interpreted as having been positive. This finding is contrary to the reports of Bizzozero and Midana [26] and Tasaki [27] who claimed to have evoked specific Frei reactions in lymphogranulomatous guinea pigs infected by the intradermal route.

We are of the opinion that the guinea pig can be used to advantage in studies of lymphogranuloma venereum. This belief is based on the regularity and readiness with which the animal can be infected with a suitable strain of virus and also on the fact that the induced disease resembles, with certain limitations, that acquired by man.

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DESCRIPTION OF PLATES

PLATE 48

FIG. 1. Guinea pig showing mass in right groin 8 days after subcutaneous inoculation of virus.

FIG. 2. Dissection of animal shown in Figure 1. Note the enlarged inguinal gland and the thickened hemorrhagic areas of attachment to the skin, thigh and abdominal musculature. The gland mass measured 2.5 by 2.5 by 0.8 cm.

FIG. 3. Microphotograph of inguinal mass 3 days after subcutaneous inoculation of virus. × 6.

FIG. 4. Microphotograph of inguinal mass 9 days after subcutaneous inoculation of virus. Compare the extent of periglandular involvement with that seen in Figures 3 and 6. × 6.

1

2

3

4

PLATE 49

FIG. 5. Four days after intradermal inoculation of active and heat-killed virus into a guinea pig.

The right groin inoculated with active virus shows an erythematous papule 12 mm. in diameter with a necrotic center 5 by 4 mm.

The left groin inoculated with heat-killed virus shows a low, slightly erythematous papule 4 mm. in diameter.

FIG. 6. Microphotograph of inguinal mass 13 days after subcutaneous inoculation of virus. × 6.

FIG. 7. Microphotograph of primary lesion in the skin 7 days after intradermal inoculation of virus. Note ulceration, acanthosis and infiltrate in the corium. × 15.

FIG. 8. Microphotograph of primary lesion in the skin showing infiltrate in corium 10 days after intradermal inoculation of virus. × 105.

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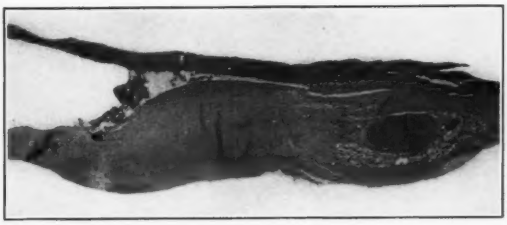

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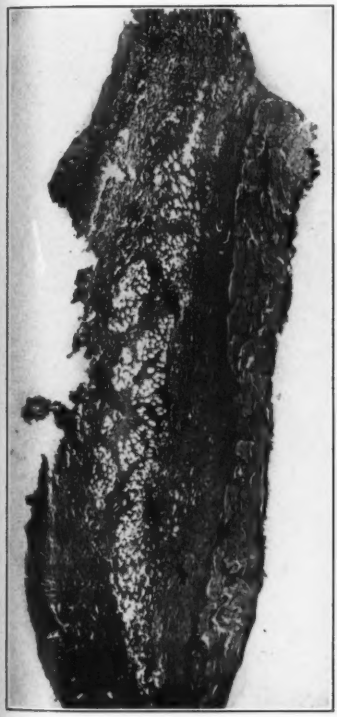

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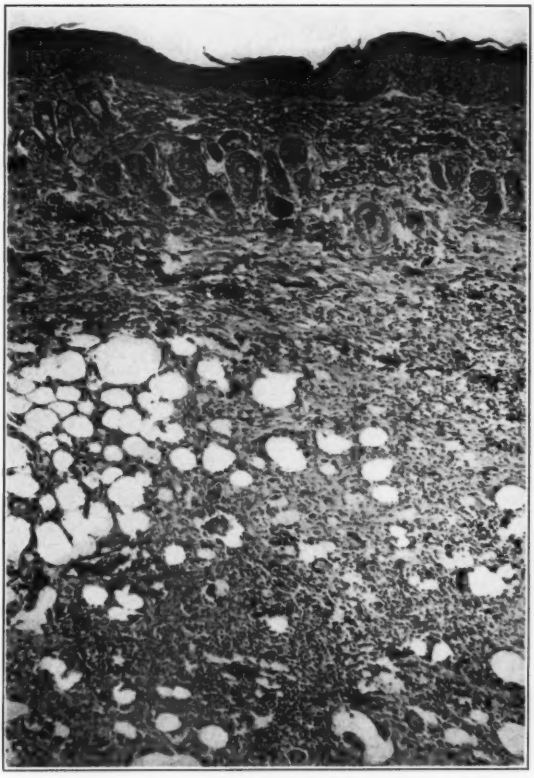

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Grace and Suskind Lymphogranuloma Venereum

PLATE 50

FIG. 9. Guinea pig presenting enlargement of penis 2 days after intrapreputial inoculation of virus. The prepuce cannot be retracted.

FIG. 10. Normal penis of guinea pig showing no interference with retraction of prepuce 2 days after inoculation of heat-killed virus intrapreputially.

FIG. 11. Swelling and ulceration of external genitalia of guinea pig 2 days after inoculation of active virus into the vaginal mucosa.

FIG. 12. Microphotograph of a portion of a cross section of penis 6 days after intrapreputial inoculation of virus. Note acanthosis and extensive distribution of hemorrhage and infiltrate. × 30.

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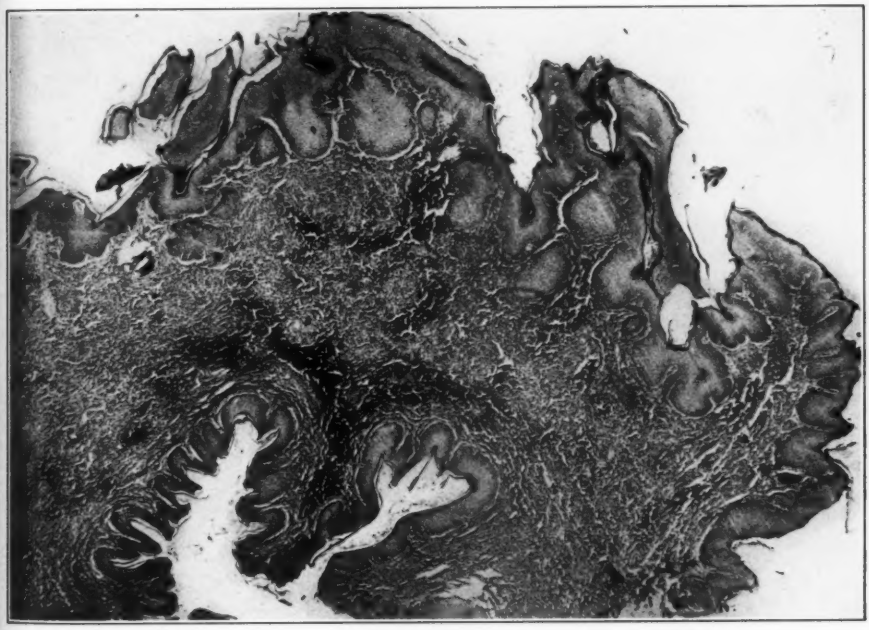

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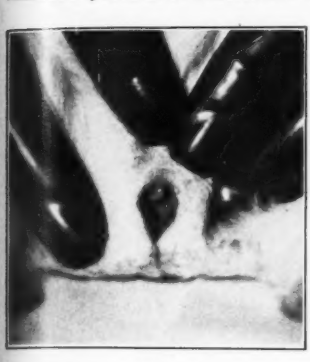
PLATE 51

FIG. 13. Normal external genitalia of a guinea pig 2 days after inoculation of heat-killed virus into the vaginal mucosa.

FIG. 14. Microphotograph of epididymis 3 days after intratesticular inoculation of virus. Note the infiltrate in the tunica vaginalis and supporting tissue. × 22.

FIG. 15. Higher power of infiltrate in the tunica vaginalis shown in Figure 14. This was the typical cellular response to inoculation of virus. × 1200.

FIG. 16. Reactions of lymphogranulomatous individual to guinea pig glands used as Frei antigen.
Uppermost = reaction to normal gland (control).
Middle = reaction to gland removed 3 days after inoculation with virus.
Lowest = reaction to gland removed 6 days after inoculation with virus.



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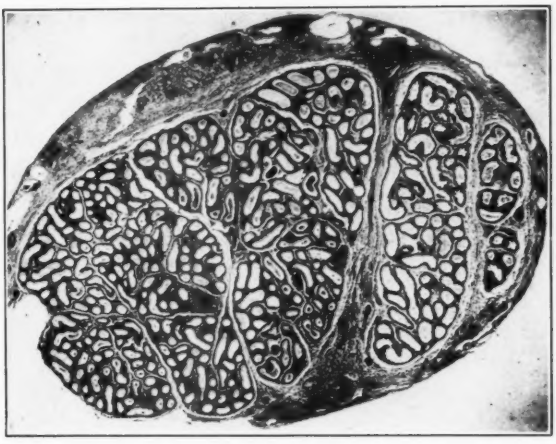

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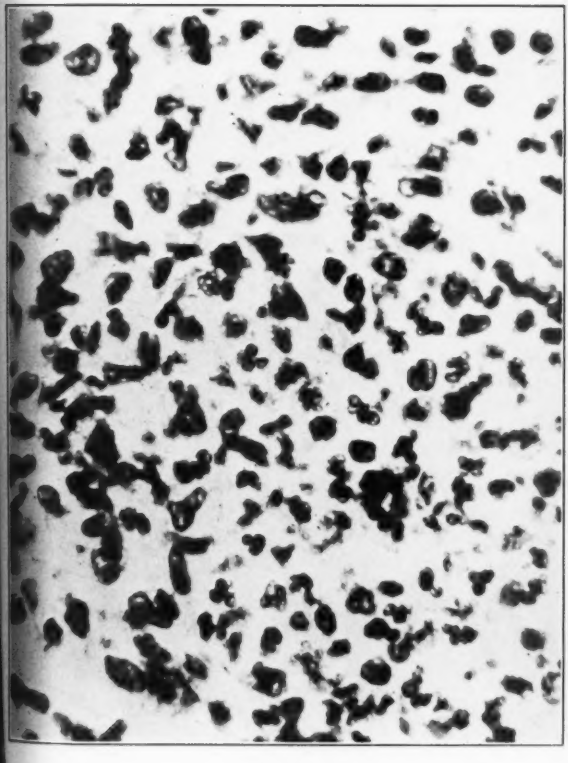

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Lymphogranuloma Venereum

INTRACYSTIC PAPILLOMA OF THE BREAST *

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Intracystic papilloma of the breast is one of the less common and perhaps least understood tumors of the breast for the reason that intracystic papilloma was and often still is confused with other lesions of the breast, all of which are agglomerated and classified symptomatically as "bleeding nipple." Today it is fully realized that what has been called "bleeding nipple" may be either an intracystic papilloma, a papillary carcinoma of the duct, or so-called chronic cystic mastitis. Since microscopic intraductile papillomas often constitute an integral part of so-called chronic cystic mastitis, it is clear that "bleeding nipple" is the result of intraductile cellular proliferations, either in the form of a true tumor, papilloma or carcinoma, or merely in the form of cellular reactions as seen in certain cystic diseases of the breast.

In the following report only the intracystic papillomas which are found as individual, single or multiple tumors in breasts showing no other changes will be discussed. Neither the above mentioned microscopic papillomas of chronic cystic mastitis nor definitely papillary carcinomas fall within the scope of this study. The gross and microscopic appearance of these tumors will be described and a classification attempted based on their histological structure. The multiplicity of these tumors and their possible relation to carcinoma will be considered and an attempt made to prognosticate possible recurrence.

The references to the literature are not complete. Only the seemingly more important studies are mentioned, particularly in so far as they are concerned with the histological description of papilloma and a discussion of its possible relation to malignancies.

REVIEW OF LITERATURE

Billroth [1] in 1860 examined 150 tumors of the breast, 4 of which were classified as cystosarcomas. He believed that the latter

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tumors arise from so-called adenoid sarcomas in which the formation of cysts occurs secondarily. From his description it seems evident that these cystosarcomas are papillomas of the breast. He also discussed the likelihood of these glandular structures in cystosarcomas being true tumors, new formations of glandular structures, or merely evidence of hyperplasia. He further stated that the cystosarcomas occurring in young individuals, as a rule, do not recur after removal. However, if they occur in older individuals they not only may recur but may also become malignant.

Leser [2] in 1887 reported 6 cases of papilloma. He coined the term "cystoma papilliferum mammae" instead of using the expression "cystosarcoma." He remarked that the epithelial proliferation and the formation of slender papillae were the important constituents of the tumor and compared this tumor with the papilliferous cystadenoma of the ovary.

Greenough and Simmons [3] in 1907 stated that papillary cystadenomas are not uncommon and that these tumors are localized, either single or multiple, and involve as a rule the large ducts of the breast. They consist of one or more cysts partially filled with papillary outgrowths arising from the wall. The papillary growths have a vascular branching connective tissue stalk supporting a luxuriant growth of epithelium in the form of villus projections and glandlike interlacing tubules and canals. The epithelium shows no tendency toward infiltrating the surrounding tissue. These authors further pointed out that tumors of this class have been recognized and described by many writers under many names: adenoma, villus papilloma, duct papilloma, duct cancer, cystadenoma intercanaliculare, carcinoma Villeux, endocanalicular papillary fibroma, and so on. They also reported 20 cases, 3 of which presented adenocarcinomas. In 12 cases the tumors were single and 5 showed different nodes in the same breast. The axillary lymph nodes were not involved, even in the carcinomas. On microscopic examination the papillary masses were often divided into many branches and occasionally interlaced, presenting the appearance of a multitude of gland tubules and ducts lying in the fibrous stroma. The epithelium lining the papillae was flattened, often consisting of several layers. The epithelial cells varied in size from low cuboidal cells of the ducts to high columnar, well formed cells seen in the adenomatous proliferation of abnormal involution.

The epithelium lining the tumor was continuous with the lining of the cyst. In the 3 cases of adenocarcinoma of the duct, the low malignancy of this tumor was especially emphasized.

Krompecher [4] in 1916 described papillary tumors in the breast supposedly the result of displaced axillary sweat glands within the breast.

Lewis [5] stated in 1916 that the reports of earlier observers indicated that the papillary cystadenomas were often regarded as duct and villus carcinomas. He emphasized that if they were carcinomas there had been relatively few recurrences even after incomplete operation.

Pribram [6] in 1921 stressed the fact that papillomas are often multiple. He emphasized the difference between papillomas which appear in the form of true tumors and papillary excrescences occurring in so-called chronic cystic mastitis.

Bloodgood [7] in 1922 stated that there is no evidence that these lesions (papillomas) are precancerous and that the prevailing view that the woman with a discharge from the nipple should be protected from carcinoma by a removal of the breast is based on fear and not on fact.

Sistrunk [8] in 1922 pointed out that the bloody discharge from the nipple, even if no tumor could be demonstrated, has often been proved to have been caused by a papilloma in one of the ducts and that surgeons generally recognize this condition as an indication for a simple amputation of the breast. He contended that these papillomas often become malignant and that as they are largely multiple, a simple amputation is the procedure of choice.

Miller [9] in 1924 was not impressed by the necessity of relying in instances of discharging nipples upon the kind of discharge, but rather on the gross and microscopic appearance of the tumor.

Hart [10] in 1927 described the gland-like arrangements of the papilloma in places, which may be attributed to the manner of cutting the sections or to the fact that the various papillae have become adherent and fused with the loss of the epithelial cells at the point of fusion. He further pointed out that these tumors have been regarded as especially liable to malignant degeneration. However, he stressed that certain factors strongly suggest that the tumors which show malignant changes are carcinomas from the

onset. In his material there was only 1 case with a history suggestive of carcinoma developing in a benign tumor.

Knoflach and Urban [11] in 1928 reported 15 cases of papilloma. They stated that the most common cause of hemorrhage from the nipple in their series was an intraductile papilloma. On 11 occasions circumscribed tumors were found close to the nipple. The tumors consisted of papillary excrescences with a loose vascular stroma lined by one layer of cylindrical epithelium. In the 4 other instances the papillary tumors were multiple. In some of these there was a more solid proliferation of epithelial cells filling out the entire lumen. These authors did not believe that such changes indicated a precancerous condition. There were no atypical cells present and a perforation of the basal membrane was not noted. However, the histological difference between individual duct papillomas is important because of the possibility of malignant changes occurring in some of these papillomas. They further stressed that so-called malignant degeneration is more likely to occur in diffuse papillomas, particularly in those which extend into the smaller ducts. Although not stating means of differentiation, they believe that it would be important to differentiate among the benign papillomas those in which malignant degeneration might occur.

Adair [12] in 1930 reported 100 cases of bloody discharge from the nipple. Of these cases 47.2 per cent showed evidence of malignancy while benign lesions were found in 52.8 per cent. He stated that certain papillary adenomas had only begun to invade, making it extremely difficult for the pathologist to classify them as malignant or benign. He stressed that papillary cystadenomas predominated among lesions producing bloody discharge. If it be true that these lesions will progress to the carcinoma stage, one would expect more instances of papillary cystadenocarcinoma than any other single type of carcinoma. This, the author believed, proves to be the case. He also stated that benign papillary cystadenomas eventually develop into papillary cystadenocarcinomas.

Lepper, Baker and Hartog [13] in 1933 described 28 benign papillomas, 4 intraductile carcinomas and 8 microscopic papillomas with diffuse proliferation of the epithelium of surrounding ducts which were suggestive of malignancy. These authors de-

fined papilloma of the breast as a tumor springing from the wall of a duct and confined within its wall. Its size, shape and position may vary and it may have one or more attachments to the wall of the cystic space in which it lies. It is composed of a connective tissue core covered by epithelial cells. Tumors of the intracanalicular type in which the connective tissue predominates were not included.

Weber [14] in 1934 reported a case of papillary cystadenoma. Histologically the lining cells were rather irregular but no atypical cells were found. The basal membrane was absent. The author referred to this tumor as a precancerous condition.

Cheatle and Cutler [15] in 1931 stated that papillomas are not consistent in their maintenance of the benign state and that their growth is definitely and commonly associated with the termination in carcinoma. They also stressed that papillomas are much more common than generally supposed. They may be present with other lesions in the breast and when their presence is not in the least suspected. They are more commonly multiple than generally supposed. To infer that only one papilloma exists in the breast that has been only partially examined is a totally unwarranted assumption. These authors give a detailed gross and microscopic description of papilloma. They classified papillomas from a morphological point of view as tumors deriving from the subepithelial connective tissue and those originating in the pericanalicular and periacinous connective tissue, and attempted to distinguish papillomas arising in the ducts from those arising in the acini. They also differentiated between uniradicular papillomas and multiradicular papillomas, which are thought to result from fusion of uniradicular papillomas in the early stages.

This survey of the literature reveals the insufficiency of histological studies of these tumors. It shows how little is known concerning their pathogenesis and finally reveals the discrepancy in opinion as to whether these tumors signify so-called precancerous conditions or not. Although the multiplicity of these lesions is often stressed, a plausible explanation of this occurrence is not given.

Material and Methods

Examination of 58 papillomas forms the basis for this study. In general, not only the papillomas but the breast tissue surround-

ing the growth was studied. In some instances microscopic sections of the whole breast, including the papilloma, were made according to Cheatle's method because it was soon realized that study of the surrounding breast tissue is important in the evaluation of the pathogenesis of these tumors. A number of papillomas were cut serially. The sections were stained with hematoxylin and eosin or with iron hematoxylin. Elastic stains (orcein and Weigert's method) and the Van Gieson's stain were employed when deemed necessary.

Gross Observations

The gross appearance of these tumors was quite characteristic. They were best demonstrated when the larger ducts of the breast were dissected open with the scissors. The ducts containing the tumors were often dilated and revealed a hemorrhagically discolored liquid. As a matter of fact, this material may be used as a signpost for the discovery of the intraductile tumors. In the region of the grossly recognizable papilloma the ducts were invariably cystically dilated. The tumors in this series varied in size from about 0.3 by 0.5 cm. to 4 by 5 cm. in greatest dimensions. Often they formed well defined localized tumors, but at times they extended in snake-like fashion into several ducts. The region of attachment of the tumors to the duct wall could be made out in every instance although it was sometimes difficult to locate. The tumors occasionally showed only a very thin pedicle which broke off easily. In other instances the tumor had a broader attachment to the duct wall. When more ducts were dissected after a large papilloma had been located, small particles of the papilloma, apparently broken up tumor tissue, were found occasionally distally or proximally to the primary tumor. In 30 instances more than 1 papilloma was present in the same breast.

The duct in the region of the papilloma was uniformly enlarged. The tumor itself when palpated through the intact duct was of a soft, almost fluctuant consistence. After opening the duct the tumor bulged and it was always difficult to close the duct after it was once incised. The diameter of the tumor seemed larger than the diameter of the duct at this site. This was always demonstrated clearly in cross sections through breasts which harbored papillomas, where these tumors seemed to "pop out." The duct

often was very thin and gave the impression of a capsule. However, close inspection invariably revealed, in some region at least, a minute space between the tumor and what appeared to be a capsule, thus definitely proving the apparent capsule to be the duct wall.

The papillomas varied in appearance, color and size. Most commonly they were 1 to 2 cm. in greatest diameter. They were yellow, somewhat translucent and presented either a smooth or fine velvety surface. Often relatively large portions of the surface of the papilloma were smooth while the neighboring regions were finely granular. Sometimes the tumor seemed to consist of several, thin, smaller friable parts held loosely together by an insignificant stalk. Other tumors were of a more reddish yellow color and presented gross evidence of hemorrhage. By dissecting several ducts it could be demonstrated in some instances that a papilloma arising in one duct extended into several adjacent branches of this duct. Most commonly the surface of these tumors was seemingly similar to the surface of the uninvolved duct. Thus, the suggestion presented itself on gross examination that the papillomas had arisen just beneath the lining of the duct, pushing the latter toward the center or opposite wall of the duct. It was noted especially that as a rule the larger tumors were confined to the duct from which they sprang while the smaller tumors extended into the adjacent ducts.

Microscopic Examination

When sections of the papillomatous tumors were studied microscopically it soon became evident that although macroscopically alike, these tumors could be classified histologically into three more or less distinctive groups. It also was soon realized that the first group of papillomas again could be subdivided into three subgroups.

Group I. Comprising this group are the vast majority of papillomas. There were 42 representatives among the 58 papillomas included in this study. All these tumors revealed definite fibrous elements in the form of connective tissue stalks which seemed to constitute the most pertinent factor. The connective tissue stalk varied in several of the tumors of this type. The appearance of the stalk formed the basis of the subgrouping of this type of papil-

loma. Most commonly it was thin and contained only a few nuclear elements and small thin walled blood vessels running through the length of the stalk. Usually several long and thin stalks were present, the tumor forming many large excrescences. The tips of such excrescences were often in close approximation and the epithelial cells of covering adjacent stalks fused. This fusion could easily be observed in serial sections. Because of this fusion the lining epithelial cells of the stalks often simulated a tubular arrangement. In instances where the papilloma consisted of many stalks which had fused, it was often difficult to decide whether the tubular structures present were true acini or tubules of the type to be described below, or whether they were formed by fusion of neighboring excrescences. However, when serial sections were made, the origin of such pseudotubular structures could often be demonstrated. Furthermore, it could be shown that the epithelial cells lining the cyst in which the tumor arose were identical with those lining the pseudotubular structures and those covering the stalks.

The epithelial cells lining the stalk were invariably very regular and showed no noteworthy morphological variations. Mitotic figures were very rarely encountered and atypical mitoses were never observed. Although basal membranes were sometimes absent, histologically these lesions were perfectly benign. The duct and the cystic structures in which these tumors arose often showed at least two layers of cuboidal lining cells, a superficial row of rather high cuboidal epithelial cells, and a deeper layer consisting of low, sometimes flattened epithelial cells. Although the epithelial cells lining the stalks of these tumors were continuous with those lining the cysts, it was often noted, as just mentioned, that only the superficial cuboidal lining cells were present on the stalks in these regions. From the above it seems clear that this type of papilloma, because of its principal constituent, may be referred to as a fibropapilloma. However, because of the fact that microscopically the pseudoglandular structures constituted the outstanding observations, this variety of fibropapilloma may be termed pseudoglandular fibropapilloma.

Rarely the stalks of some of these tumors were broad and consisted of much hyalinized connective tissue with few blood vessels and sparse cellular elements. The individual papillae often re-

mained separate from one another, fusion did not occur readily and pseudoglandular structures were not found. Although also belonging to the group of fibropapilloma, these tumors may be subclassed as acellular fibropapilloma.

Occasionally papillomas undoubtedly also belonging to this group were found which consisted of broad and rather long stalks which were very cellular. The cells were oval or spindle shaped, with little cytoplasm and small oval nuclei with dust-like chromatin particles. Often these cells were spread by an edema-like material. Multinucleated cells were often encountered and a few mitotic figures could easily be detected. The epithelial cells covering these papillae were similar to those described above. The papillae were occasionally so broad that the space between two papillae was obliterated and two long rows of cells were often found extending through the length of the papillae. Here and there only small pseudotubular structures were present seemingly intercalated in the course of the rows of epithelial cells. This tumor may be classed as the cellular variety of the fibropapilloma. It seems possible that this type represents an early stage of the acellular fibropapilloma. Combination forms of pseudoglandular papilloma and either cellular or acellular fibropapilloma were occasionally encountered.

From the above it seems clear that the papillomas described represent three forms of one type of papilloma. The one common factor in these tumors is apparently the extension of connective tissue from outside the duct into a dilated duct or cyst. The connective tissue forms the bulk of these tumors in the form of stalks which are still covered by the duct-lining cells. For this reason the term "fibropapilloma" is used. Depending on the constituents of the stalk, two forms can be distinguished, the cellular and the acellular fibropapilloma. For those instances in which branches of the stalks fuse and form pseudoglandular structures, the term pseudoglandular papilloma may be appropriate.

Group II. Another type of papilloma, grossly distinguishable from the fibropapilloma only by the slightly firmer consistence of the latter, consisted microscopically primarily of true glandular structures. Among the 58 papillomas 9 were identified as belonging to this group. These glandular structures varied in size but were as a rule small and showed distinct basal membranes. They

were lined by one layer of uniform, rather high cuboidal cells. Occasionally groups of acini were found within this tumor which consisted of pale staining cells larger than those lining other acini and showing clearly outlined cell membranes. Atypical mitotic figures were not observed. The stroma here was scant and often absent, the glandular structures predominating. It could often be shown that groups of the glandular structures which formed the tumor and extended into the cystic duct were covered by another type of cuboidal cell which in every respect was similar to that lining the duct. Often, however, only the more superficial duct-lining cells covered the tumor. This epithelium could always be distinguished from that forming the glandular structures of which the tumor consisted. In every instance it could be seen clearly that extensions of the duct-lining cells separated groups of glandular structures from one another with the formation of small or larger excrescences of the tumor. Occasionally it was noted that this tumor was not confined to one duct but extended in the form of thin ramifications into other ducts. The covering duct epithelium still was observed occasionally in these instances, although more often it was absent. Because of the principal constituent of this papilloma, it may be referred to as glandular or adenomatous papilloma.

Although a number of papillomas were of the true adenomatous type, others consisted of both the fibrous and the adenomatous types. In such tumors true glandular structures and pseudotubular structures as described above were found side by side.

The glandular type of papilloma was also characterized by a number of small sized blood vessels which were found beneath the epithelial lining of the glandular excrescences which were continuous with the lining of the duct in which the tumor apparently arose. These vessels were often dilated and filled with red blood corpuscles. Since rupture of the walls of these vessels was occasionally noted, it seems clear that they were the source of the bloody discharge from the nipple which clinically is so characteristic of these papillomas.

Sections which were cut from the whole breast showed that in the region of the papilloma, outside the duct or cyst from which the tumor arose, often a large number of glandular structures similar to those of which the papilloma consisted were noted.

These glandular structures did not resemble adenomas but rather islands of hyperplastic acini more or less concentrated about the cystic structure. In serial sections it could be shown that the hyperplastic glandular structures extended from the outside into the cyst. The epithelial lining cells of the cyst gradually became invaginated and were pushed deep into the lumen of the cyst. In other words, it seemed that glandular structures arising outside the cyst secondarily grew into the dilated ducts and formed these glandular papillomas, their intracystic part still being covered by the original duct or cystic epithelium. Within the duct more glandular structures were formed extending in various directions within the cyst or dilated duct. Thus, end portions of the tumor were often found at a great distance from the region where the glandular structures originally had extended into the duct.

In a number of sections taken from the ducts of the breast at a distance from the tumor small glandular structures similar to those of which the tumor consisted were found either free in the lumen or apparently attached to the lining cells of the duct. The cells of these glandular structures showed no evidence of degenerative changes and their nuclei seemed perfectly normal. From the sections examined it could not be stated definitely whether these structures constituted an artefact and were displaced from the tumor by instrumentation, or whether they had broken loose *intra vitam* and had lodged in other parts of the duct.

GROUP III. The third type of papilloma again could not be distinguished grossly from the two just described. It was perhaps of still softer consistence than the glandular type. Microscopically it was quite different since there were practically no glandular or pseudoglandular structures present, nor was there a pronounced stalk. Seven tumors of this type were encountered. This tumor consisted principally of epithelial cells which either seemed to arise from the lining cells of the duct or from a very thin inconspicuous stalk. Particularly in early stages it could be shown that initially there was a heaping up of epithelial cells at one or several portions of the duct forming sessile growths. The thin connective tissue stalk seemed to be formed secondarily. The epithelial cells of this papilloma varied somewhat in shape. They were either polyhedral, round or oval, and often assumed a more spindle shape, only the basal cells of these sessile tumors being cuboidal. When

the spindle shaped cells predominated the tumor appeared to be more of a transitional cell type. In many fields of individual papillomas of this type the transitional-like cells predominated. The nuclei in these tumors were rather small, round or slightly oval and vesicular, with distinctly outlined nucleoli. When a connective tissue stalk was present it contained a more centrally located blood vessel and the cells seemed to come off the stalk in the form of sheets. In these instances the tumor closely resembled a transitional cell papilloma of the urinary tract. In several sections it could be shown that in contradistinction to the two other varieties of papilloma the lining cells of the duct did not cover this tumor. In some sections more than in others the tumor cells seemed similar to the basal cell layer of the lining cells of the duct. Rarely early proliferations of lining cells of the duct were observed where the resemblance of these cells to spindle cells of the tumors was very clear.

In some instances the tumor cells had completely occupied the cystic spaces and had also extended into ramifications of the primarily involved duct. However, the duct walls were always recognized and though they sometimes seemed absent, further sectioning revealed that these portions corresponded to the branching points of ducts. In some sections which were taken from the vicinity of this type of papilloma, transitional cells similar to those of which the tumor consisted were found either free in the lumens of other ducts or on normal appearing, duct-lining cells where they seemed to have lodged after being shed from the tumor. In other words, some of the tumor cells seemed to be implanted on other portions of the duct. From the appearance of the cells of which this tumor consists it may be designated as transitional cell papilloma. Occasionally fibropapillomatous or glandular papillomatous structures were found in an apparently primarily transitional cell papilloma.

Discussion

From the microscopic study of tumors of the breast which grossly were designated as papillomas, it seems clear that three types of this tumor may be distinguished: the fibrous (pseudoglandular), glandular, and transitional cell papilloma. It was also shown that combinations of these types do occur, particularly of

the fibrous and glandular types. The fibrous type is characterized by an extension of periductile connective tissue into the duct at various portions. Thus, the lining epithelium is pushed toward the lumen of the duct by the connective tissue growth and seems to be invaginated. The fibropapilloma again may be subdivided into three groups: (1) the type consisting of broad, fibrous, practically acellular excrescences lined by one layer of epithelial cells. The excrescences here remain as a rule isolated and do not fuse (acellular fibropapilloma). (2) The type consisting of broad, very cellular and often edematous excrescences, also covered by duct-lining cells. The epithelial cells of adjacent papillae are often in such close approximation that two papillae are separated only by two layers of epithelial lining cells (cellular fibropapilloma). (3) The type consisting of slender long papillae lined by duct-lining cells. The tips of these papillae almost invariably become adherent to adjacent tips and form pseudoglandular structures which are the most prominent feature of this type (pseudoglandular fibropapilloma). It seems likely that the proliferation of connective tissue here is the primary factor and that it is of secondary importance that the connective tissue extends into the duct, giving rise to the formation of papilloma. Because the duct-like spaces into which the connective tissue extends are larger than ducts and visible to the naked eye, these spaces should be designated as cysts.

As the result of the approximation of two or several slender excrescences, pseudoglandular structures are formed which can be distinguished from true glandular structures by the similarity of their lining cells to those lining the duct or cyst respectively. In not a single instance of this type of papilloma were any changes noted which could be interpreted as possibly precancerous in nature. All these tumors appeared morphologically perfectly benign and as far as could be ascertained did not recur. From this study it seems that the majority of papillomas of the breast belong to this group.

The glandular type of papilloma consists of true glandular structures which are similar to the acini of the breast. In serial sections it could be shown that primarily there is a hyperplasia of the acini or perhaps a true adenoma surrounding a large duct. Secondarily these adenomatous structures impinge upon and ex-

tend into the walls of cysts without actually invading the latter but pushing the cyst wall and lining cells into the lumen. Thus it happens that groups of intracystic glandular structures are lined by the duct- or cyst-lining cells. In larger papillomas and in those extending into smaller ramifications the lining cells may be absent. The stroma in these papillomas is much less pronounced than in the fibrous type. As mentioned above, combination forms of the pseudoglandular and the true glandular type often occur. Because of the type of epithelium, it is often relatively easy to distinguish the true from the pseudoglandular structures.

The fact that glandular or papillomatous structures are found in contact with extracystic or extraductile tissue was noted by Cheatle and Cutler.[15] However, these investigators believed that the extracystic tissue was invaded secondarily by the papillomatous structures. Yet they stressed that these structures are perfectly benign. From our studies it seems that the glandular structures have primarily proliferated outside the ducts or cysts and secondarily extended into the cysts forming the papillomas.

The multiplicity of these tumors is striking, but is perhaps less common than the multiplicity of the transitional type. Too few tumors of the latter type were encountered to permit a more exact statement. Often a similar genesis of the multiple papillomas could be demonstrated, namely an extension of hyperplastic pericystic acini into the cyst. Sometimes, however, the breast tissue surrounding smaller papillomas showed no changes. In these instances it may be possible to explain the multiplicity of these tumors by implantation of tumor cells on normal lining cells. This view is based on the finding of loose tumor cells free in the lumen and the presence of these cells adjacent to the lining epithelial cells in the ramification of the duct involved. This will be referred to later.

Occasionally in these papillomas glandular structures were found which were lined by pale staining (hematoxylin-eosin) lining cells which were somewhat larger than those lining the acini. Their cytoplasm was well outlined, their nuclei small, slightly hyperchromatic and situated at the base of the cells. These cells are often referred to as "blasse Zellen" and their origin, according

to Krompecher,[4] can be traced to misplaced sweat glands which are supposedly identical with those seen in the axilla. Tumors, *i.e.*, mainly cysts lined by these cells, are therefore referred to as "hidrocystomas." Occasionally hidromatous papillomas could be recognized which showed characteristics similar to those of the simple glandular papillomas.

The third type of papilloma apparently arises from the lining cells of the duct. Although not generally agreed, it seems from this study and as stated by some investigators (Maximow and Bloom,[16] and Schaffer [17]) in contradistinction to Cheatle and Cutler,[15] that the ducts are lined by a layer of stratified cuboidal cells. Often two rows of cells are clearly recognizable. It seems that the uppermost layer of cells which lose their high cuboidal shape gives rise to the papilloma in question, since in early instances one or two rows of more cuboidal cells were still seen forming the basal cell layer, whereas the more superficial cells were more oval, round or transitional in type. These transitional cells resemble transitional cells of the urinary bladder and hence this type of papilloma resembles those seen in the urinary bladder and perhaps certain papilliferous cystadenomas of the ovary. Only a little stroma is seen in these tumors and the stalk is insignificant. This type of papilloma is apparently never a single tumor, but often several similar papillomas are found throughout the breast. Occasionally desquamated tumor cells are found situated on normal appearing, lining epithelial cells. Because of this finding and of the morphological resemblance between this tumor and some ovarian tumors, it seems possible that the multiplicity of these papillomas can be explained on the basis of implantation of tumor cells. It is known that benign papillary cystadenomas of the ovary may occasionally produce implantation metastases on the peritoneal surface (Baer [18]). Such metastasis is not a true indication of malignancy but can be compared to tissue culture growth and is sometimes referred to as tissue culture metastasis. Although it is quite possible that the multiplicity of these papillomas represents an example of true multiplicity of a primary tumor, from the facts presented it also seems possible that these multiple lesions can be explained on the basis of one primary papilloma with multiple implantations.

It should be mentioned in this connection that Cheatle and

Cutler [15] described sessile growths consisting of epithelial cells which are pressed together, lose their columnar shape and become cuboidal, oval and round or polyhedral in shape. Their nuclei are hyperchromatic and mitoses may be present, although there is not the slightest indication of their transgression of former boundaries. It seems clear that these authors refer to early transitional cell papillomas.

There is quite a divergence of opinion in the literature in regard to the possibility of papillomas of the breast undergoing "malignant degeneration." As a matter of fact, papillomas of the breast are regarded by some investigators as precancerous lesions and radical mastectomy is advocated by Klose [19] in every instance of bleeding nipple. From this study, however, it seems clear that neither the fibrous nor the glandular papilloma is a tumor which is at all suspicious of malignant potentialities. In not a single instance of these tumors were there cytological changes encountered which could be interpreted as either precancerous in nature or of questionable early malignancy. In other words, these two types of papilloma of the breast are perfectly benign tumors morphologically, and as far as subsequent histories of these patients reveal, they show no recurrence although only the tumor was removed in some instances.

The transitional cell papilloma of the breast, which in this study is the least frequently encountered type of papilloma, however, is more difficult to evaluate. In studying the microscopic sections of this tumor one is inadvertently reminded of a papilloma of the urinary bladder and of certain ovarian tumors. The resemblance to the latter was especially pointed out by Leser [2] and also by Kaufmann.[20] Just as it is difficult, from the morphological point of view, to state definitely whether certain papillomas of the urinary bladder are malignant tumors or not, the same may be said for some of the breast papillomas of the transitional cell type. The recurrence of these papillomas in the bladder is a well recognized fact. Because of these difficulties the transitional papillomas of the urinary bladder are often classified as carcinomas, Grade I (Barringer [21]). As mentioned before, there also are papilliferous cystadenomas of the ovary which, although of perfectly benign morphological appearance, are capable of producing implantation metastases on the peritoneal surface (Baer [18]). From

this study it seems that all these considerations must be applied to the transitional cell type of papilloma of the breast.

Two illustrative cases which are not included in this series may be cited. In two instances where a papilloma of this type had been removed, the patients were readmitted to the hospital after periods of 1 and 2½ years respectively. In the first instance, at second admission another papilloma of the same type was found at the operation, and in the second instance, a duct carcinoma. In the first instance it seems that there was *a priori* more than one papilloma, which at the time the first papilloma was removed was perhaps too small to have been noted clinically. This explanation is probably correct since the second tumor was not found in the line of the operative scar. From this study and from the literature it is clear that papillomas often are multiple. It is also known and it has been found in this study that in some instances papillomas were found on gross sectioning of breasts which had been removed for hemorrhage from the nipple and in which clinically no tumor was palpable. So it seems likely that in this case the other papilloma might have been present at the time of the first operation. Whether the presence of more than one papilloma indicates a true multiplicity of origin or may be explained on the basis of implantation metastases from one primary papilloma, as mentioned above, cannot be decided, of course.

In the second instance the tumor at first glance may be interpreted as "malignant degeneration" or transformation of a benign papilloma and may be taken as a characteristic example by those who believe that papillomas of the breast must be regarded as a precancerous condition. While proof for such an assumption cannot be offered, it is at least possible to explain this occurrence in two other ways. Just as the papilloma of the bladder is considered as carcinoma, Grade I, although morphologically it seems to be a benign tumor, so perhaps the transitional cell type papilloma of the breast may be considered an anatomically benign tumor in which malignant characteristics are not recognizable by means of the present day methods of examination. In other words, these tumors may be *a priori* malignant tumors, although histologically they may appear benign. These papillomas may, therefore, be classified as carcinomas of a low grade of malignancy. The other possible explanation presented itself after the examination of a

breast which was removed for carcinoma. On sectioning, four tumors could be identified. Three of these were papillomas of the glandular variety and one was a typical carcinoma simplex of the breast. There was, as far as could be determined, no relation whatsoever between these papillomas and the carcinoma. It seems that the carcinoma occurred in a breast that happened to show papillomas instead of in a normal breast. In other words, the carcinoma was found coincidentally with other tumors in the same breast. Instead of too readily assuming a transitional cell papilloma of the breast to be a precancerous lesion, and instead of asserting a so-called "malignant degeneration" of a benign tumor, these two other possibilities must be carefully evaluated.

From the foregoing, the application of this anatomical study seems clear. The fibrous or pseudoglandular and the glandular papillomas are benign tumors in every respect, although they may be multiple. Because of their multiplicity it is perhaps wiser not only to excise the tumor but to remove a circumscribed region of breast tissue surrounding the tumor. The prognosis in these cases should be very good. There is no danger whatsoever that carcinoma may develop on the basis of these lesions. In case of a transitional cell papilloma of the breast, it seems that simple mastectomy is indicated because of the possibility that these lesions are perhaps, just as are the papillomas of the urinary bladder, tumors which histologically appear benign but which may possess an inert degree of malignancy not recognizable by our present day staining methods. After mastectomy the prognosis in these patients is good.

The differences in the histological pictures of the individual papillomas of the breast, and particularly the fact that there are several types of papilloma, are perhaps responsible for the conflicting opinions expressed in the literature in regard to their possible malignancy and to the surgical procedure involved in their removal. An attempt to classify these papillomas histologically has apparently not been made previously. Thus it seems possible that various investigators had different types of papillomas in mind and apparent conflicting statements may be explained by the possibility that various authors examined the different types of papillomas. Adair's [12] statement that benign papillary cystadenomas eventually develop into papillary cyst-

adenocarcinomas may refer to the transitional cell type of papilloma. The same explanation may be offered for the opinions held by Sistrunk,[8] who stated that these papillomas often become malignant. Klose,[19] as mentioned above, advocated radical mastectomy for all instances of bleeding mammae, including of course, all types of papilloma. Sauchelli [22] stated recently that many papillomas are associated with carcinoma. Also, Cheatle and Cutler [15] stressed that papillomas are not so consistent in their maintenance of the benign state and that their careers are definitely and commonly associated with the termination in carcinoma. On the other hand, Erdheim,[23] Risak [24] and others were against the assumption that papillomas should be regarded as precancerous. However, in the 2 cases Risak reported it seems that the papilloma was definitely of the glandular variety. Knoflach and Urban,[11] realizing that all papillomas do not fall into the same classification, stated that malignant degeneration is more likely to occur in instances of diffuse papillomas which extend into the smaller ducts. It seems clear that this refers most likely to the transitional cell type of papilloma. They also stated that they would like to separate entirely benign papillomas from those in which "malignant degeneration" might occur.

So it seems that a careful histological examination of these papillomas of the breast is essential and that the surgeon should not be satisfied with the diagnosis of "papilloma." Grossly it seems impossible to distinguish the various types of papilloma. Thus, histological classification is important, not only from the anatomical standpoint but, more important, as a guide to the surgical procedure and as it concerns the prognosis for the patient.

Summary and Conclusions

Histological investigation of 58 intracystic papillomas of the breast reveals three distinct varieties of these tumors: a fibrous type, a glandular type, and a papilloma consisting of cells which closely resemble the transitional epithelium of the urinary bladder and hence may be designated as the transitional cell type. The fibrous type consists of a stalk of connective tissue which often is very thin. Ramifications of the stalks may fuse with the production of pseudoglandular structures. This is the most common type of papilloma. Because of such pseudoglandular structures, this

special subgroup of the fibrous variety may be designated as the pseudoglandular type. The glandular type is apparently formed by an extension of neighboring hyperplastic or adenomatous periductile acini into a duct or cyst, the epithelial cells of the duct enclosing the invaginated acini. Both the fibrous and glandular types are benign tumors which do not recur. They often extend into the neighboring ducts but cannot be regarded as precancerous. The transitional cell type of papilloma morphologically is a benign tumor. However, it may recur after removal and some of the recurrent tumors may show morphological evidence of malignancy. It resembles the papilloma of the urinary bladder which, although morphologically benign, is sometimes classified as carcinoma Grade I principally because of recurrences which prove to be malignant. Intracystic papillomas of the breast are as a rule multiple. The multiplicity of these transitional cell papillomas may be explained on the basis of multiplicity of origin or by implantations of tumor cells in neighboring ducts. Such implants do not necessarily indicate malignancy but may be compared to certain benign ovarian tumors which occasionally produce implantations on the peritoneal surface.

This study shows the importance of careful histological examination of all papillomas of the breast as far as the prognosis for the patient is concerned and also in regard to the surgical procedure. In instances of fibropapilloma and glandular papilloma, not only the excision of the tumor is indicated but also the removal of a circumscribed region of the breast surrounding the tumor, because of the fact that often these tumors are multiple. There is no danger that carcinoma may develop on the basis of these lesions. In instances of transitional cell papilloma of the breast, simple mastectomy is indicated because of the possibility that these lesions are perhaps tumors which, although histologically benign, may possess an inert degree of malignancy not recognizable by our present staining methods. After mastectomy the prognosis in these patients seems good.

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DESCRIPTION OF PLATES

Plate 52

Fig. 1. Fibropapilloma. Note the presence of only a few cellular elements within the papillae. Iron hematoxylin preparation. × 50.

Fig. 2. Fibropapilloma, cellular variety. Note the many cellular elements within the broad papillae. The epithelial lining of two neighboring papillae are in close approximation. Iron hematoxylin-eosin preparation. × 75.

Fig. 3. Pseudoglandular variety of fibropapilloma. Iron hematoxylin preparation. × 50.

Fig. 4. Pseudoglandular variety of fibropapilloma. Iron hematoxylin preparation. × 130.

Fig. 5. Glandular papilloma. Note the difference in the lining cells of the papillae and those of the glandular structures. Hematoxylin-eosin preparation. × 120.

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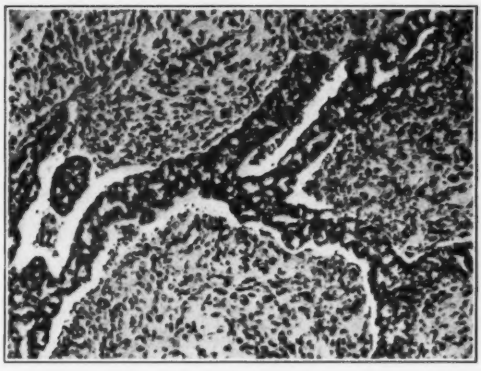

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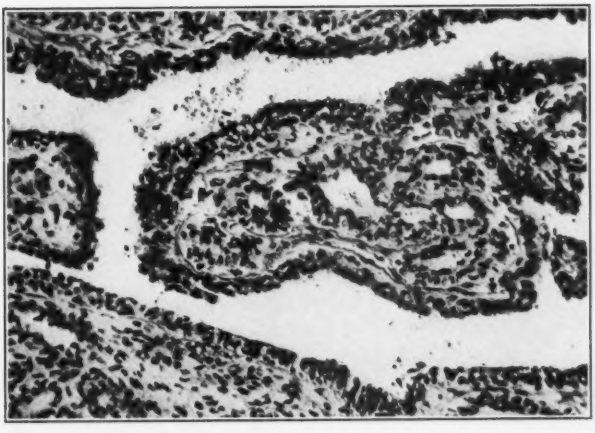

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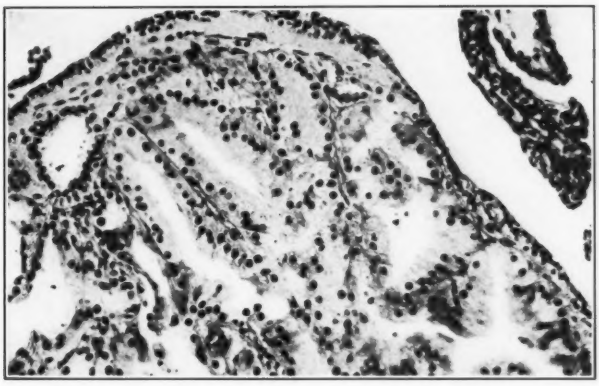
Plate 53

Fig. 6. Glandular papilloma. Note the pale staining lining cells of the glandular structures ("blasse Zellen"). Hematoxylin-eosin preparation. × 130.

Fig. 7. Glandular papilloma. Note the cuboidal lining cells of the papilloma. Hematoxylin-eosin preparation. × 50.

Fig. 8. Early transitional cell papilloma. Note the difference between the basal cell layer and the more superficial layers. Hematoxylin-eosin preparation. × 110.

Fig. 9. Transitional cell papilloma. Note the spindle shaped epithelial cells of the tumor. Iron hematoxylin preparation. × 120.



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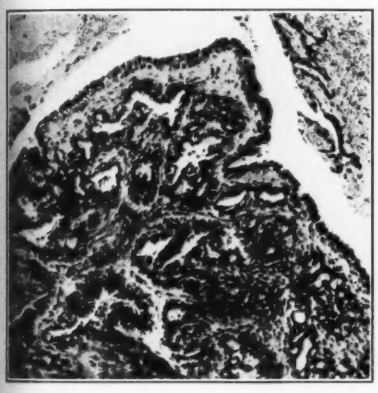

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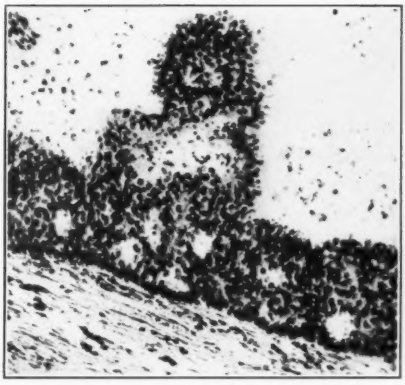

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Saphir and Parker Intracystic Papilloma of Breast

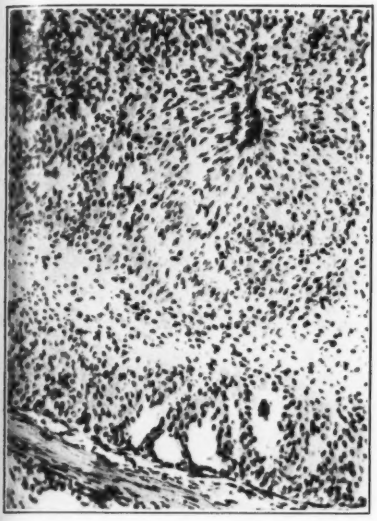
PLATE 54

FIG. 10. Transitional cell papilloma. Note the transitional type of epithelial cells and the small vessels in the right upper half from which the cells seem to radiate. Iron hematoxylin preparation. × 95.

FIG. 11. Transitional cell papilloma. Note the similarity of this tumor to the transitional cell papilloma of the urinary bladder. Iron hematoxylin preparation. × 95.

FIG. 12. Glandular and transitional cell papilloma. Iron hematoxylin preparation. × 120.

FIG. 13. Extension of fibropapilloma from one duct into another. Iron hematoxylin preparation. × 80.



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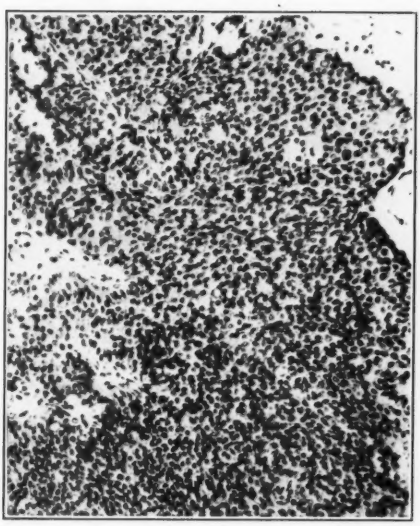

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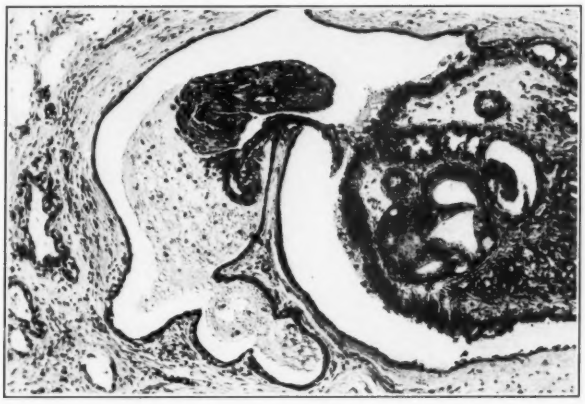
PLATE 55

FIG. 14. Extension of papilloma into a neighboring duct. Note the small connecting pedicle. Iron hematoxylin preparation. × 75.

FIG. 15. Hyperplasia of acini close to a cyst wall. Hematoxylin-eosin preparation. × 70.

FIG. 16. Loose tumor cells in cyst (artefact ?). Iron hematoxylin preparation. × 100.

FIGS. 17 and 18. Plaques of tumor cells apparently implanted upon duct-lining cells. Iron hematoxylin-eosin preparation. Fig. 17, × 50; Fig. 18, × 225.



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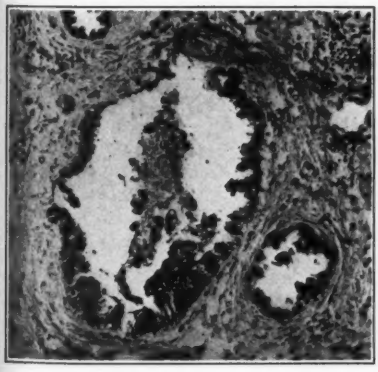

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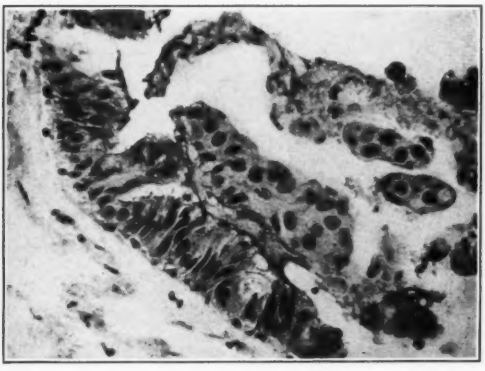

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Saphir and Parker

Intracystic Papilloma of Breast

THE PATHOLOGICAL ANATOMY OF CELLOPHANE PERINEPHRITIS *

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In the course of experiments designed to prevent the development of collateral circulation to the kidneys following renal arterial clamping,[1, 2] it was found that the application of cellophane to the kidneys produces an intense inflammatory reaction around them, and that in 3 to 4 weeks sustained hypertension appears.

It is the purpose of this communication to report in detail the pathological changes in a group of 10 dogs following the application of cellophane to one or both kidneys.

MATERIAL AND METHODS OF STUDY

The dogs were anesthetized, a kidney exposed, freed from its bed, and after gently stripping off the fat from its surface cellophane was wrapped around the kidney and secured either with punch paper clips or by loose, all-embracing ligatures around the pedicle, without compression. It is not essential that accurate approximation of the cellophane around the kidney should occur. The kidney was replaced and the wound closed. This procedure was repeated, if desired, on the opposite kidney. Rabbits and cats also have been used successfully in experiments of this type.

The blood pressure was determined by direct arterial puncture on the femoral artery.

OBSERVATIONS

Table I presents the essential clinical and postmortem observations on 10 dogs subjected to the operation. The arterial pressure may begin to rise as early as the 14th day after operation, and becomes sustained at levels of 180 to 238 mm. Hg. The longest

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TABLE I

Number of dog	Experimental procedure	Postoperative survival period	Blood pressure before operation	Initial rise in blood pressure postoperatively	Average level of blood pressure postoperatively	Maximum level of blood pressure	Renal changes
			mm. Hg.	*day*	*mm. Hg.*	*mm. Hg.*	
397	Cellophane both kidneys	2 days	122	..	..	..	Acute perinephritis Parenchyma intact
392	Cellophane both kidneys	7 days	126	..	..	..	Acute perinephritis Early fibrous deposit, parenchyma intact
282	Cellophane left kidney; right nephrectomy	14 days	122	..	150	..	Subacute productive perinephritis Parenchyma intact, no compression of pedicle discernible
379	Cellophane right kidney	31 days	130	20th	188	238	Acute and chronic productive perinephritis Parenchyma intact, no compression of pedicle discernible
350	Cellophane both kidneys	6 wks.	113	14th	202	226	Subacute and chronic productive and constrictive perinephritis Focal cortical tubular atrophy, ischemic type of scarring, fresh infarction in one kidney
389	Decapsulation and cellophane both kidneys	2½ mos.	120	24th	170	179	Chronic perinephritis Focal microscopic scarring of ischemic type, compression (?) of pedicle, non-occlusive organized parietal thrombus, left renal vein
355	Cellophane both kidneys	3 mos.	115	14th	190	232	Chronic productive and constrictive perinephritis Marked compression both kidneys and pedicles, recent and old infarcts in one

362	Cellophane left kidney; right nephrectomy	4½ mos.	129	15th	176	222	Chronic productive and constrictive perinephritis Marked rotation of kidney, with compression of pedicle, focal cortical tubular atrophy and scarring
339	Cellophane right kidney and left kidney	4 mos. 5 mos.	118	28th	194	214	Chronic productive and constrictive perinephritis Microscopic scarring in left, pedicle compressed in scar tissue, artery patent on both sides
340	Cellophane both kidneys; right and left adrenalectomy	6 mos. 1 mo. 1 wk.	117	19th	180	200	Chronic productive and constrictive perinephritis Marked compression and focal cortical scarring, both pedicles compressed, organized non-occlusive thrombi in renal veins

observations have been made in animals surviving operation for 13 months.

At postmortem examination the kidneys in short experiments are found surrounded by a sanguineous purulent exudate (Fig. 1) forming a membrane between the true capsule and the cellophane. A similar exudate may be found on the outer aspect of the cellophane, and the omentum is likely to cover the whole zone.

After 2 weeks the exudate around the cellophane may still be sanguineous and purulent, but a dense fibroblastic and collagenous deposit appears on the true renal capsule (Figs. 2 and 3). This increases to a thickness of 3–5 mm., extends around the entire kidney, and may or may not be reflected on the structures of the pedicle.

Involvement of the pedicle in the earliest experiments produced no apparent constriction. Adhesions between the omentum and some of the pedicle structures led to no apparent distortion. After 6 weeks, constriction of the pedicle, notably the vein and the ureter, becomes discernible.

At the end of 2 to 3 months the constrictive effect is visible (Figs. 5 and 6) and the kidney may be rotated in any plane, with more or less compression of the cortex and the hilar structures. The collagenous hull which forms external to the normal capsule is readily separated from it and the true capsule may show little or no change. It, in turn, may be readily stripped from the underlying parenchyma. In the early stages the cellophane remains intact. After 2 to 3 months the cellophane may be crumpled and displaced toward one pole, or broken into several pieces caught between the dense fibrous deposits. When removed it appears to be unaltered, and a shreddy, fibrinous purulent exudate may still be present on it. Small fragments which are crumpled may be found enclosed within dense fibrous scars, but there is no evidence of intracellular digestion or phagocytosis. There is remarkable lymphoid hyperplasia in the lymph nodes of the omentum, and these regularly exhibit acute lymphadenitis.

The omentum becomes more firmly attached (Fig. 5) to the exudate around the external aspect of the cellophane, and dense, richly vascularized adhesions form. Where the cellophane lies close to the spleen (Fig. 6) on the left, or the liver on the right, the capsules of these organs may participate in the inflammatory

process. The same productive reaction may occasionally be found around the adrenal gland.

Histological examination of the kidney reveals negligible changes in the parenchyma for the first 2 months. Later, and throughout the period of observation thus far used, marked compression of the cortex with focal cortical and tubular atrophy (Fig. 3) and cortical scarring of the ischemic type becomes apparent. On the capsule there is a mixture of blood elements and fibrin (Fig. 1) which at the end of 7 days is almost replaced by the rapidly proliferating young connective tissue (Fig. 2) and granulation tissue. When there is marked distortion and compression of the kidney, small cortical scars may be visible on close examination (Fig. 5), but microscopic study is usually necessary to detect these zones. The glomeruli are remarkably well preserved in most instances. In some the hilar tips of the renal poles frequently show deeper and more diffuse scars than are seen elsewhere.

As far as we can tell the cellophane remains intact (Fig. 4), suffering no change other than fragmentation during its residence in the tissues. Where the cellophane is broken up, small dense nodules may form around the fragments, but these remain extracellular. In these nodules and in the hyperplastic lymph nodes found in the omentum, the histological changes include polymorphonuclear leukocytic infiltration and mobilization of large numbers of macrophages containing large quantities of brownish pigment usually forming an amorphous mass in the cytoplasm of the cells. Hemosiderin granules may also be found in an intracellular position. The stimulation to fibroblasts is conspicuous in these nodules until the collagen condenses and the cells disappear.

The following protocol is an example of an experiment in which the pedicle as well as the kidney was involved, and where death was due apparently to renal insufficiency.

Dog No. 362: This animal was found dead in its cage 4½ months after a right nephrectomy and the application of cellophane to the left kidney. The blood pressure had been persistently high (Chart 1). The blood urea nitrogen was 281 mg. per cent the day of death, and there was severe diarrhea during the day. The clinical course until the day of death had not been remark-

able, and the animal had withstood its operation without any untoward reaction.

Postmortem examination revealed the following significant changes. There were about 200 cc. of free blood in the peritoneal cavity. The peritoneum itself was clear and glistening. The omentum was brownish and wrapped around a mass in the left side of the abdomen. Included within the mass and bound by

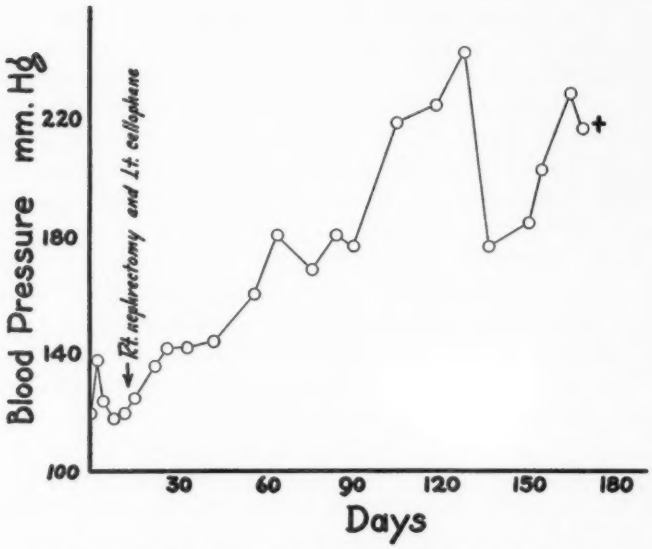

CHART 1. Graphic chart of blood pressure observations in Dog 362.

dense adhesions to the omentum and to each other were the spleen, the left kidney and left adrenal (Fig. 6). Lymph nodes in the omentum were enlarged and brownish. The other organs exhibited nothing remarkable.

On dissection of the mass the left ureter appeared to be displaced upward. It passed to the level of the superior portion of the kidney and then made a sharp right angle turn toward the kidney with the renal artery. Both structures were traced through the attached omentum.

On removing the omentum a crumpled piece of cellophane was found beneath and in a pocket of dense collagen at the upper pole.

Further separation showed that the kidney was surrounded by a dense mantle of connective tissue. The pelvis was found embedded within dense connective tissue and the renal artery could be traced into the same tissue; at the hilum it was impossible to tease the renal artery away from the scar. A loose string ligature was found around the pedicle, not constricting any structures.

A longitudinal section of the kidney toward the hilum revealed marked upward rotation of the kidney (Fig. 6), resulting in upward displacement of the pelvis previously noted. The true renal capsule could not be seen but a dense deposit of collagen covered the cortex and was inextricably bound to it. There were no visible areas of infarction or hemorrhage.

Cross sections of the hilar structures showed marked compression of the renal artery and the ureter. The renal vein could not be discerned.

There was a small amount of hemorrhagic fibrinous exudate on the surface of the splenic capsule, which on section was found to overlie a fresh area of hemorrhagic infarction. There were no other gross changes of interest, except that the heart appeared moderately enlarged (weight 167 gm.) and hemorrhages were found in the papillary muscles of both ventricles.

Microscopic Examination

Intestine: Normal. No degenerative vascular lesions. The walls of some of the arterioles appear hypertrophied.

Stomach: The submucosal vessels exhibit medial hypertrophy and some narrowing of their lumens. This section includes an area of hemorrhage into the muscularis which separates the muscle bundles and extends irregularly toward the submucosa. There are foci of interstitial histiocytic proliferation and of polymorphonuclear leukocytic accumulation in the muscle. No acute necrotizing vascular lesions are encountered. In this section the hemorrhage is also visible under the peritoneum. *Diagnosis:* Arteriolar sclerosis and hypertrophy, and multiple hemorrhages into the muscularis.

Spleen: There is moderate arteriolar medial hypertrophy. The pulp appears normal. There is a moderate amount of hemosiderin stored in the macrophages.

Another section of the spleen, through the zone of infarction noted on gross examination, reveals an early hemorrhagic infarct in which the red cells are still intact. There is a fibrinous purulent exudate on the splenic capsule overlying the zone of infarction. Several foci of liquefaction necrosis are apparent in the depth of the infarct and great numbers of leukocytes are present in these zones. The trabecular vessels are swollen and their lumens virtually obstructed by the edematous walls. No thrombi are encountered in this section. *Diagnosis:* Infarction of spleen.

Omental Lymph Node: Marked hyperplasia of the pulp with intense infiltration by polymorphonuclear leukocytes and large numbers of brownish macrophages is seen. The latter are encountered in the sinuses of the lymph node as well.

Adrenal Gland: The adreno-cortical arterioles exhibit marked medial hypertrophy and some show hyalinizing changes. All show narrowing of their lumens. There are no parenchymal changes. *Diagnosis:* Arteriolar sclerosis.

Myocardium: The myocardium is the seat of widespread focal necrosis and infarction. Hemorrhages are most marked beneath the endocardium and extend for a considerable distance into the muscle. The vessels uniformly exhibit medial hypertrophy and narrowed lumens, but no thrombi are encountered in the vessels studied. *Diagnosis:* Multiple miliary infarcts.

Aorta: The outer third of the media exhibits zones of cystic separation of the medial fibers. Similar, less conspicuous areas are encountered in the inner third. The vasa vasorum are normal.

Kidney: The surface of the kidney is the seat of a dense fibrous proliferation replacing the true capsule. In places there are vascularized adhesions to fragments of the omentum. At several points there are deposits of fibrin and extravasation of red cells.

The parenchyma is fairly well preserved; the glomeruli are recognizable, but there are many wedge shaped zones of corticotubular atrophy (Fig. 3) and replacement fibrosis. These are uneven and extend irregularly toward the pelvis. In some there are calcific deposits in the lumens of the affected tubules. There is a considerable amount of brownish pigment deposited in the epithelial cells of the collecting tubules. In the middle of one of the medullary pyramids there is a focus of polymorphonuclear

leukocytic infiltration in the interstitial tissue and in the lumen of the tubule. The blood vessels show no intimal or medial changes. Some appear compressed and irregular in outline.

Hilar Structures: Two cross sections were prepared of the hilar structures. One includes the hilar poles of the renal cortex and shows extensive fibrosis and replacement of the tubules. The glomeruli for the most part are still remarkably well preserved. The renal artery and pelvis are embedded in a dense mass of connective tissue which is the seat of a rich leukocytic and histiocytic infiltration. The smaller arteries are obviously compressed by this connective tissue. The pelvis (or ureter) is also compressed. The supporting tissue is loose and the muscle fibers appear separated by edema fluid. *Diagnosis*: Chronic perinephritis, focal cortical scarring of the ischemic type, focal atrophy, marked compression of the pelvis and ureter, and compression of the kidney.

Sections of the other organs revealed no significant changes.

Discussion

It should be emphasized that hypertension occurs when the fibrocollagenous hull surrounds only the parenchyma of the kidney. Several dogs have been alive for over a year with hypertension, and their blood urea is normal; hence renal insufficiency is not a necessary concomitant of the hypertension.

The *early* development of hypertension following the application of cellophane could not be related to visible compression of the renal vessels or ureter, even when no special care was taken to ensure that the cellophane did not come in contact with the pedicle. The *later* development of scar tissue around the structures of the renal pedicle may well contribute to renal ischemia. However, compression of the parenchyma alone is necessary to produce hypertension. It is difficult to say how much renal ischemia is present during the stages of acute perinephritis immediately following the application of cellophane, but it seems unlikely that it is severe. Certainly the renal artery and its branches were not narrowed in experiments up to 1 month.

The response to cellophane in these experiments has been duplicated in other parts of the body where cellophane has been

used. In unpublished experiments studies have been made of the reaction to cellophane placed on the liver, around the intestine, and around the adrenals. It has also been placed in the subcutaneous tissue. There is a similar macroscopic and microscopic reaction to its presence in each of these situations.

Cellophane and cellophane-like substances are unusual, as far as we know, as sclerosing agents, in that they do not disappear, exhibit strong chemotactic stimulation of polymorphonuclear leukocytes, and induce a dense fibroblastic reaction with collagen formation, lasting at least as long as there are cells and vascular channels around the cellophane to permit the reaction. This material should prove useful in fascial reconstruction, and in other situations where a dense collagenous response is desired.

Hypertension produced by this method occurs whether the normal capsule is stripped or not before the application of cellophane; denervation of the kidneys also does not interfere with its development.[1] Removal of a cellophane-wrapped kidney from animals in whom hypertension has followed the application to one kidney causes the hypertension to disappear, provided it has not persisted for a long time.

Summary and Conclusions

1. It has been found that sterile cellophane, when gently applied to the kidneys of dogs, rabbits and cats, produces an intense inflammatory reaction and strong chemotactic stimulation of polymorphonuclear leukocytes, followed by a continuous fibroblastic and collagenous deposit.

2. The collagenous deposit may be 3–5 mm. thick, and after 1½ to 2 months the renal parenchyma or hilar structures, or both, may be compressed.

3. The systolic blood pressure begins to rise 3 to 4 weeks after the application of cellophane, and may reach a level of 240 mm. Hg. after a month or two. It has persisted for as long as 14 months.

4. The pathogenesis of this type of hypertension appears to be related to the perinephritis; it is difficult to ascribe it to ischemia, on morphological grounds, since there are no ischemic changes in the kidneys in the early stages. Further studies of the blood flow through the affected kidneys are necessary to determine the occurrence of ischemia in this type of experiment.

5. In later stages ischemic scarring is easily discernible, and compression of the kidney probably interferes with capillary circulation. Likewise there may be marked compression of the hilar structures by the scar tissue.

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DESCRIPTION OF PLATES

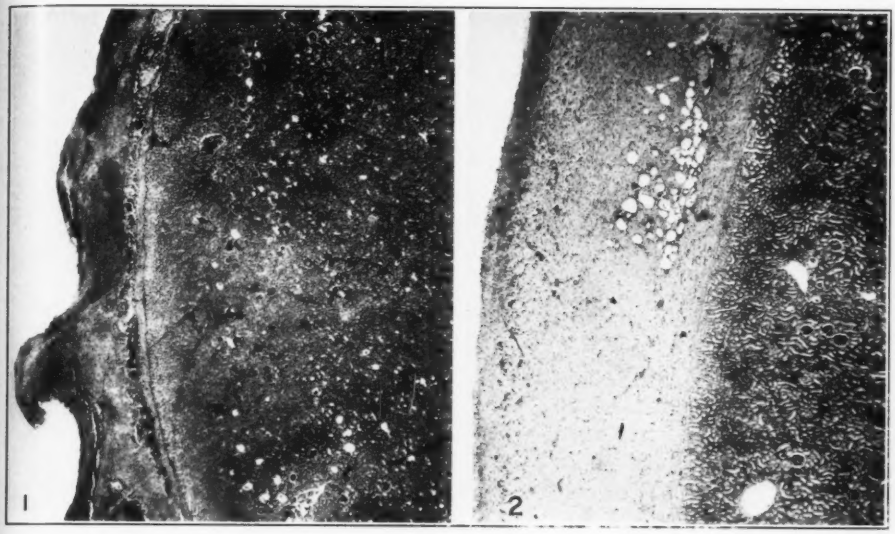
PLATE 56

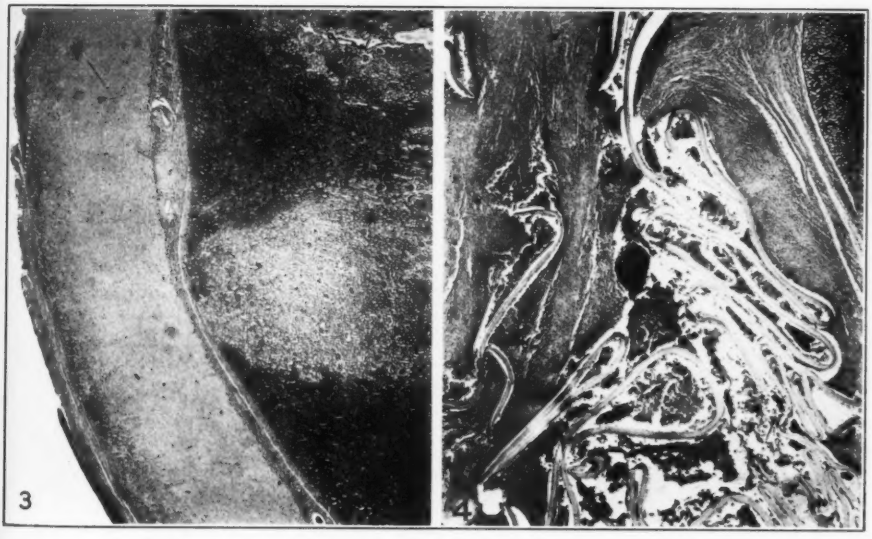
FIG. 1. Section of a kidney 2 days after the application of cellophane showing the rich fibrinous purulent exudate on the surface of the true capsule of the kidney. The parenchyma is wholly intact.

FIG. 2. Section including the renal cortex and the rich fibroblastic and collagenous deposit laid down on the true capsule of the kidney 2 weeks after the application of cellophane. Near the surface can be seen young granulation tissue and the persistent fibrinous purulent exudate.

FIG. 3. Section of the kidney shown in Figure 6 showing wedge shaped zones of tubular atrophy separated by a zone of normal parenchyma.

FIG. 4. Section through a kidney and the surrounding fibrocollagenous hull 6 months after the application of cellophane. Note the persisting purulent exudate around the crumpled cellophane. Fragmentation of the cellophane is apparent in the scar tissue at the arrow.



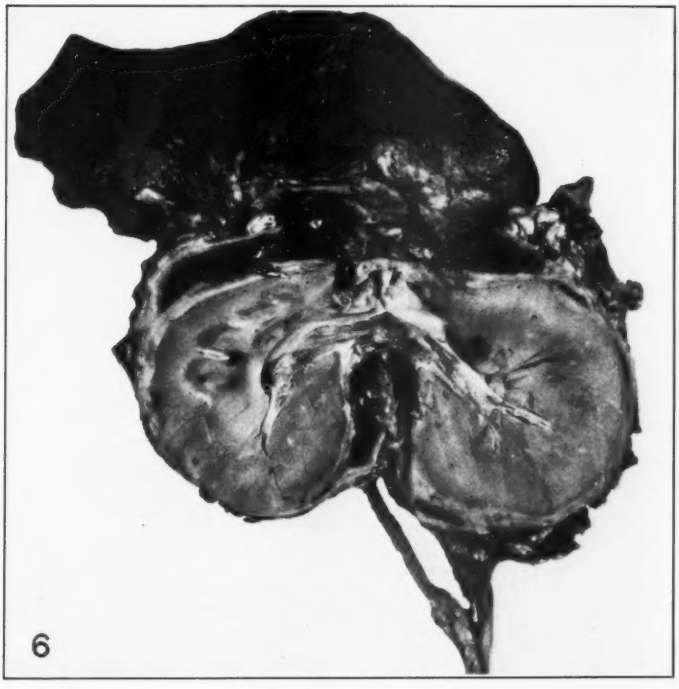


Graef and Page Cellophane Perinephritis

PLATE 57

FIG. 5. Longitudinal section of a kidney embedded in and compressed by connective tissue. The cellophane can be seen crumpled in a pocket at A, and a portion of the omentum is attached by adhesions at various points in the zone of inflammation. Note the constrictive effect on the form of the kidney (3 months after the application of cellophane).

FIG. 6. A view of the left kidney and attached spleen in Dog 362 4½ months after right nephrectomy and application of cellophane to the left kidney. The cellophane can be seen crumpled in a pocket of connective tissue at the superior pole of the kidney. The kidney has been rotated upward, with marked compression of the organ and the structures of the pedicle.



Graef and Page Cellophane Perinephritis

HISTOGENESIS OF HEPATIC CIRRHOSIS IN CHRONIC FOOD SELENOSIS *

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The material to be described herein was derived from the experiments previously reported by one of us,[1] in which the experimental procedures were fully outlined. The seleniferous, low protein, high carbohydrate diet No. 25 produced a nodular cirrhosis of the liver with ascites and anemia. Tissues from 27 rats fed this diet for varying periods of time were removed for study as follows: 6 after 14 days, 7 after 35 days, 5 after 55 days, 5 after 74, 77, 83, 83 and 104 days respectively, 3 after 126 days, and 1 after 143 days.

At the end of the first 2 weeks the liver was normal (2 rats), slightly congested (2 rats), or showed in 1 a few hyaline fibrinoid capillary thrombi, and in another scattered, isolated, coagulated necrotic liver cells which appeared to grade into coarse hyaline oxyphilic globules. Scattered mitoses were seen in 1 liver.

After 5 weeks 1 rat's liver showed only a patchy, fine fat droplet accumulation in midzonal liver cells, 3 showed scattered coagulated liver cells and hyaline globules, and the remaining 3, early cirrhotic changes either focally (1) or diffusely distributed (2). These changes consist of irregular capillary congestion which grades into hemorrhage, often periportal in location, and in 1 rat restricted to a few areas. The hemorrhagic foci may contain scattered clumps of liver cells and regularly show proliferation of fibroblasts. Such organizing hemorrhage grades in turn into patches of solid fibroblastic proliferation enclosing numbers of isolated surviving liver cells and macrophages laden with hemosiderin, and marginally extending between the cells of the adjacent liver tissue. Such areas of proliferation connect with adjacent portal areas and the capsule, occasioning capsular retraction and a nodular surface. Some delicate, irregular fibroblastic trabeculation is produced. An accumulation of fine fat droplets is seen in

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the cytoplasm of liver cells abutting on hemorrhages and on trabeculae. In 1 of these rats scattered coagulated liver cells and hyaline oxyphilic globules were seen. Dilated blood spaces sometimes contained clumps of large lymphoid cells, normoblasts and megakaryocytes. Lymphocytic infiltration of fibroblastic areas was inconstant.

After 55 days all 5 rats showed periportal hemorrhages with organization and more or less interlobular or irregular trabeculation, capsular retraction and surface nodularity. Scattered liver cells with partial or general cytoplasmic oxyphilia, oxyphilic cytoplasmic granules or hyaline globules, or complete coagulation necrosis with karyorrhexis, grading into large, hyaline oxyphilic globules lying free in the hepatic cell cords or embedded in the fibrous trabeculae, are seen in most of the animals, and more frequently than before. The trabeculation now shows some tendency to be collagenous as well as fibroblastic, and fatty changes in hepatic tissue are infrequent.

Livers from the 5 rats killed in the 11th to the 15th week showed similar changes with fibrosis replacing the proliferative changes to a greater extent. Focal hemorrhage was seen in 1 rat, isolated necrotic liver cells and hyaline globules in 2, and nodular regenerative hyperplasia of the liver cells left between the trabeculae is now evident. Some areas are seen in which hepatic tissue is missing and the capillary spaces are widened to sinusoidal proportions with perhaps a few scattered surviving liver cells remaining, and sometimes fatty degeneration of bordering liver cells or even numbers of coagulated necrotic cells.

In the 3 rats killed at 126 days delicate, focally pigmented fibrous trabeculae separating rounded nodules of quite large, pale, granular liver cells with deeply staining nuclei are seen. Mitoses are sometimes numerous. Trabeculae tend to be interlobular but may also be irregular in location.

In a rat surviving 143 days there was a coarsely nodular hyperplasia of the liver cells with irregular fibrous trabeculation, slight lymphocytic infiltration and moderate pigmentation of trabeculae, capsular retraction, and a nodular surface. The liver cells showed quite marked, irregular intracellular accumulation of fat droplets, fine to coarse in size.

The pancreas was studied in 17 rats, 6 in the 14 day group, 5 in

the 35 day, 5 in the 55 day group, and 1 after 83 days. Significant lesions were absent. The stomach showed a slight eosinophilic or lymphocytic infiltration of the base of the antral mucosa or in the mucosa at the proventricular line. These findings were similar in 14 and 55 day rats, and are probably not significant.

The spleen was studied in 24 of the 27 rats. In general the follicles were of moderate size and moderately hyperplastic in all stages. Phagocytic activity of the follicular phagocytes was slight or absent in the 14 day animals and almost constantly present in moderate amount thereafter. Generally the perifollicular zones were more or less anemic and the reticuloendothelium of the pulp was relatively prominent up through the 55 day group. Thereafter this area was blood-filled, similar to the rest of the pulp. This may indicate some passive congestion. The pulp was well filled with blood throughout and often showed more or less numerous, large lymphoid and myeloid cells, erythroblasts, normoblasts and megakaryocytes. In general, this hemopoietic activity was more pronounced in the later 55–126 day rats than in the earlier 14–35 day group. This compares with the frequent anemias observed [1] in the D 25 group. Hémosiderin pigmentation was essentially negligible throughout. The bone marrow was studied in 9 rats, 4 of the 14 day group and 5 of the 55 day group. Myelopoiesis, erythropoiesis, and the fat content were essentially similar in both groups and apparently not significantly altered from normal.

The kidneys from 4 rats (55 and 126 days) showed a few calcified cortical tubules with or without surrounding basophilic regenerating epithelium. Otherwise there was only slight and inconstant, finely granular swelling of the epithelium of the convoluted tubules, perhaps accompanied by a little oxyphilic granular exudate in the lumens.

Passing to the other seleniferous dietary groups one finds cirrhotic changes in the liver less frequent and generally less pronounced, or much later. Sections of the livers from 23 rats receiving the low protein, high fat seleniferous diet No. 27, 1 each taken after 74 and 92 days, 9 after 120 days and 12 after 150 days were studied.

Among the 11 rats * exposed 120 days or less, 4 showed slight

* Nine rats in this group were negative grossly at autopsy and no sections were made.

periportal fibrosis and inconstantly, slight interlobular capsular retraction. Scattered, coagulated necrotic liver cells were seen in 4 rats and in 1 a few hyaline oxyphilic globules appeared. In 1 non-cirrhotic liver numerous mitoses were seen in the liver cells. Among the 12 rats exposed for 150 days, 2 showed slight, 2 patchy irregular, and 4 well marked diffuse cirrhosis with isolated surviving liver cells, moderate pigmentation and lymphocytic infiltration inconstantly present in the trabeculae. Isolated necrotic liver cells were seen in 1 cirrhotic and in 1 non-cirrhotic liver from rats of this group. However, these rats were an older group than the rest of the D 27 rats, and this may have had some influence.

More or less pronounced accumulation of fine and medium sized fat droplets was seen in the liver cells in 6 of the 11 animals killed at 120 days or less, and in 7 of the 12 150 day rats. However, a similar frequency of fatty change was seen in rats fed the non-seleniferous, low protein, high fat control diet No. 29. This control group showed no necrosis of liver cells and no cirrhosis.

In the high protein seleniferous diet No. 28 the livers of 12 rats * were studied, all killed on the 120–121st days. A well marked cirrhosis was present in 1 rat, and 4 showed slight to moderate interlobular and periportal fibrosis with little capsular retraction. The hyaline oxyphilic globules and scattered necrotic liver cells of the low protein seleniferous diets were absent, and fatty changes were not seen.

The second control group, receiving a low protein, high carbohydrate, non-seleniferous diet, presented no hepatic lesions.

The spleen in the low protein, high fat diet group showed follicles of normal size and degree of activity with little phagocytic activity of the follicle reticulum cells. The follicular phagocytes were, contrastingly, quite active in the high protein seleniferous diet group, much as in the later stages in the high carbohydrate group. Myeloid activity in the pulp was slight in the high fat group and absent in the high protein and both high fat and high carbohydrate control diet groups. It is to be noted that no anemia was found in these groups.[1] Both the high fat and high protein seleniferous diet groups showed a moderate pigmentation of the splenic pulp by hemosiderin and dark brown iron-free pigment. However, the high fat control diet was also

* Thirteen rats in group D 28 showed no gross lesions, and no sections were made.

associated with considerable hemosiderosis of the pulp, and as the excess carbohydrate diets showed a similar paucity of hemosiderin in the splenic pulp, with and without selenium, this pigmentation is probably not significant.

Calcified tubules in the proximal convoluted zone of the kidney were seen in 2 of 9 high fat selenium rats, in 4 of 7 high protein selenium rats, in all of the high fat control rats, and in 2 of 5 high carbohydrate controls. It is evident that this change is not due to selenium. Otherwise the kidneys showed slight to negligible cloudy swelling in all groups.

The pancreas, as before, was normal in all groups, as was the duodenum. No significant difference is seen in the stomach between the high fat and high carbohydrate seleniferous and non-seleniferous diet groups, all showing minor changes similar to those previously noted.

Summary and Conclusions

Wheat from seleniferous soil produces a well marked nodular cirrhosis of the liver when combined with a low protein, high carbohydrate diet. Associated with this are a considerable amount of cellular destruction and phagocytosis of nuclear débris in the follicles of the spleen, and a moderate splenic myelosis. High fat, low protein and high protein diets do not completely protect against these actions of organic selenium but do greatly delay and decrease their frequency.

Organizing hemorrhage is a frequent and possibly essential stage in the histogenesis of the cirrhosis of food selenium poisoning in rats. The destructive process is continuous, as shown by the presence of scattered, isolated necrotic liver cells, and of the hyaline oxyphilic globules derived from them at all the later stages in the process.

The cirrhosis produced by organic selenium poisoning does not especially resemble that of cinchophen,[2] copper,[3,4] silicic acid,[5] or phosphorus [6] poisoning.

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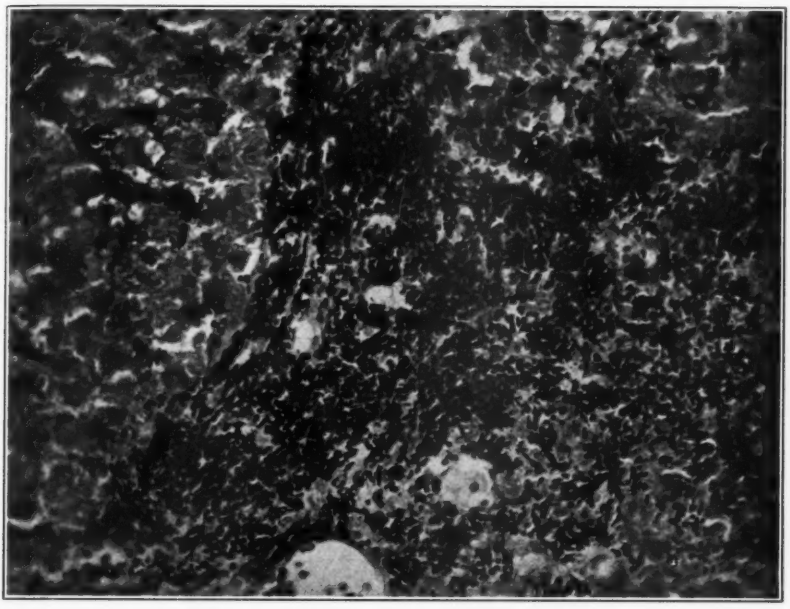
DESCRIPTION OF PLATES

PLATE 58

FIG. 1. Series D 25, Rat 15317. Early periportal hemorrhage, 35 days. Giemsa stain. × 250.

FIG. 2. Series D 25, Rat 14715. Nodular cirrhosis, 83 days. Masson fast green stain. × 125.



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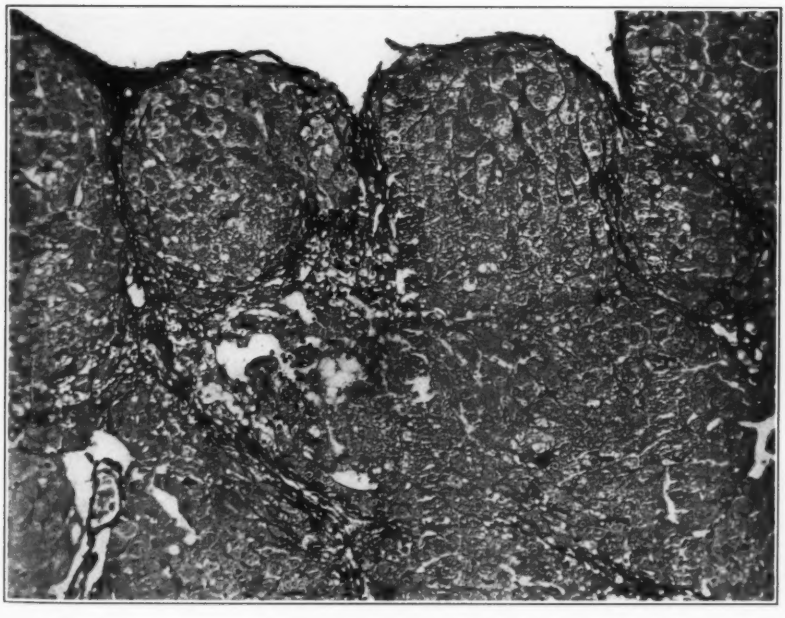
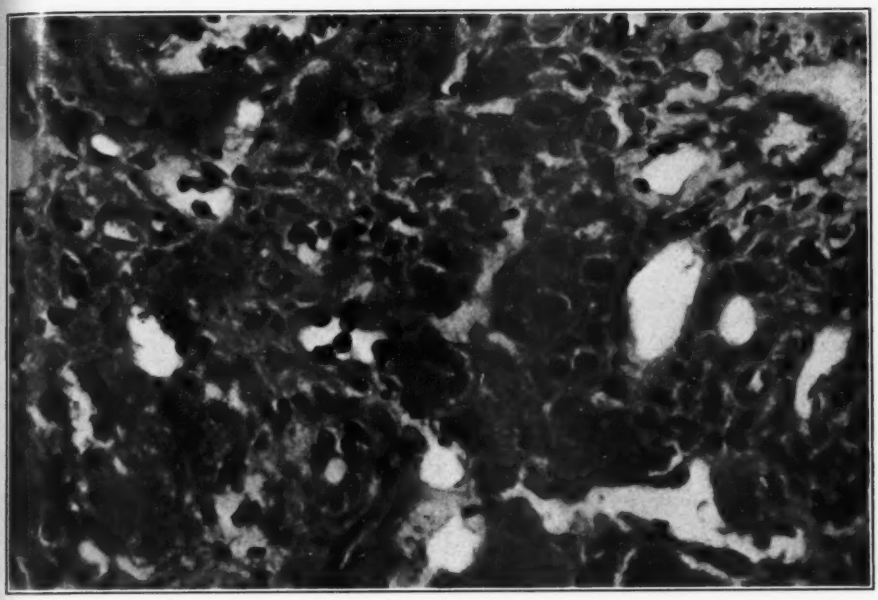


2

PLATE 59

FIG. 3. Series D 25, Rat 15317. Periportal and interstitial fibroblastic proliferation, 35 days. Giemsa stain. × 500.

FIG. 4. Series D 25, Rat 14717. This is an attempt to show varying grades of cytoplasmic oxyphilia of liver cells. The more oxyphilic cells are darker, 83 days. Giemsa stain. × 500.



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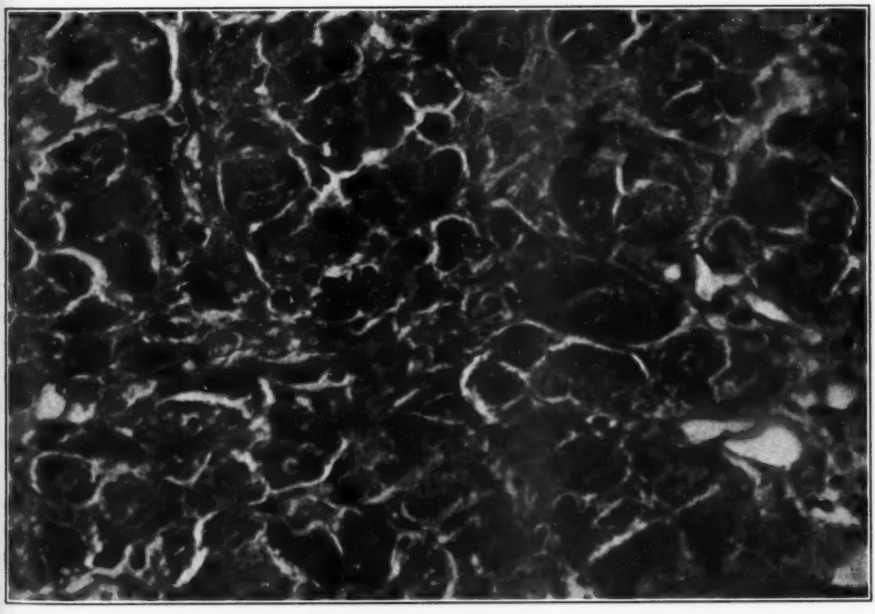

4

TUBERCULOSIS INVOLVING THE PULMONARY VALVE *

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Involvement of the epicardium and myocardium in generalized miliary tuberculosis is not unusual. Involvement of the endocardium is less common, and of the valves comparatively rare. Baker [1] in an analysis of 900 autopsies at Duke Hospital found 6 cases of tuberculosis of the endocardium, but in only 1 of these was the lesion on a valve (tricuspid). In reviewing 13,000 protocols at Johns Hopkins Hospital he found that endocardial tubercles occurred most frequently in the right ventricle at the base of the pulmonary valve.

Gross [2] reported a case with polypoid tuberculous lesions of the pulmonary artery at its bifurcation and "extending upward from a point immediately to the left of the commissure joining the right anterior and posterior cusps. A third polyp was just to the left of the base of the second polyp." The valve leaflets and commissures were normal. The patient had generalized miliary tuberculosis.

Mark [3] recently reported a case of miliary tuberculosis showing polypoid tuberculous lesions of the ventricular aspect of the pulmonary valve.

Because of its rarity the following case of tuberculous involvement of the pulmonary valve is considered worthy of note.

REPORT OF CASE

Clinical History: T. L., a colored male, aged 21 years, a C.C.C. enrollee, was admitted to the Walter Reed General Hospital May 2, 1938, and died November 4, 1938.

The past medical history was not significant and the family history revealed no tuberculosis.

The chief complaints on admission were pain in the back and fever with onset in March, 1938. The pain was in the lower dorsal and lumbar region, chiefly on the right side, and did not radiate. He had had a slight cough for a month previous to the onset of the pain and had lost about 12 pounds in weight.

Physical Examination: The significant findings on physical examination were as follows: The patient was well developed, weighed 140 pounds, and was 5 feet and 4 inches in height. Pressure over the lower dorsal spine elicited

* Received for publication August 23, 1939.

pain and flexion of the spine caused pain in this region. The erector spineae muscles were spastic.

The percussion note was impaired over the left apex; tactile fremitus and vocal resonance were increased and the breath sounds were harsh. No râles were heard before or after coughing.

A soft systolic murmur at the apex was not transmitted.

Stereoscopic examination of the chest on May 3rd revealed a dense area of infiltration in the upper left lobe, chiefly in the first interspace anteriorly. The left apex was moderately hazy and there was cloudiness of the left hilum. These findings were interpreted as old fibrous tuberculosis of the left upper lobe.

X-ray examination of the spine on May 9th was interpreted as showing tuberculosis of the body of the tenth dorsal vertebra with a paraspinal abscess surrounding the ninth, tenth and eleventh dorsal vertebrae.

Further X-ray studies on August 4th showed mottling of both lung fields, interpreted as hematogenous spread of the tuberculosis. The tenth dorsal vertebra showed partial collapse and narrowing of the intervertebral space between the ninth and tenth dorsal vertebrae. Both the ninth and eleventh dorsal vertebrae showed some destruction of the right lateral portions of their bodies. The paraspinal abscess was increased in size.

Course of Illness: During the patient's stay in the hospital he had fever varying from 99 to 101 or 103° F. Cough was slight and sputum scant. No acid-fast bacilli were ever found in the sputum.

In October he developed meningeal symptoms and the spinal fluid showed a maximum of 1920 white blood cells with 87 per cent polymorphonuclear leukocytes and 13 per cent lymphocytes. The sugar content of the spinal fluid was 46.5 mg. per 100 cc.

Postmortem Examination

The upper lobe of the left lung showed several caseous nodules from 2 to 3.5 cm. in diameter. Miliary tubercles were scattered through all lobes of both lungs. The hilus lymph nodes showed areas of caseation necrosis. Miliary tubercles were found in the liver, spleen, both kidneys, parietal peritoneum, mesentery and on the serosal surface of the ileum. The prostate showed large areas of caseation. No lesions were noted in either testis or epididymis. Surrounding the ninth, tenth and eleventh dorsal vertebrae was a large abscess. The bodies of the ninth and tenth dorsal vertebrae were soft and necrotic. Permission to open the head was not obtained but fluid withdrawn by cisterna puncture was cloudy and contained acid-fast bacilli.

The heart weighed 307 gm. The muscle was fairly firm and dark red in color. No tubercles were seen in the epicardium, myocardium or on the endocardium of the chambers. The aortic, mitral and tricuspid valves were normal. The sinus of Valsalva

of the right anterior cusp of the pulmonary valve was about half filled with a yellowish, firm, irregularly shaped mass, firmly adherent to the proximal portion of the cusp but not adherent to the intimal surface of the pulmonary artery. The distal half of the leaflet was not involved. Viewed from the ventricular aspect the valve appeared normal.

Histological Examination

Typical tuberculous lesions were found in all the organs showing gross evidence of tuberculosis.

The lesion of the pulmonary valve showed typical tubercles with central areas of necrosis surrounded by small round cells, epithelioid cells and giant cells. The process involved the valve ring but did not extend into the myocardium. The proximal half of the valve cusp was destroyed while the distal portion escaped.

Acid-fast bacilli were demonstrated in the valvular lesion and in sections of the lung.

Discussion

It is a disputed question in tuberculous lesions of the endocardium and valves whether the bacteria are deposited from the blood passing through the chambers of the heart or from nutrient vessels to the heart. In this case the lesion corresponds to the distribution of vessels supplying the valve cusp. Bayne-Jones [4] describes two sources of blood supply for the semilunar valves. One is from the vasa vasorum of the pulmonary or aortic artery. These vessels enter the valve at the point of attachment of the valve to the artery and penetrate the valve a short distance along the line of closure. The other source is from vessels of the auricular endocardium which form a plexus in the base of the cusp and extend outward half the width of the cusp. This lesion conforms to the distribution of this second group of vessels.

Summary

A case of tuberculous involvement of the pulmonary valve in a colored male, aged 21 years, who had advanced tuberculosis of the prostate, dorsal vertebrae and lungs, with a terminal miliary dissemination, is reported.

NOTE: The author desires to express appreciation to Colonel George R. Callender for his helpful suggestions in the preparation of this report.

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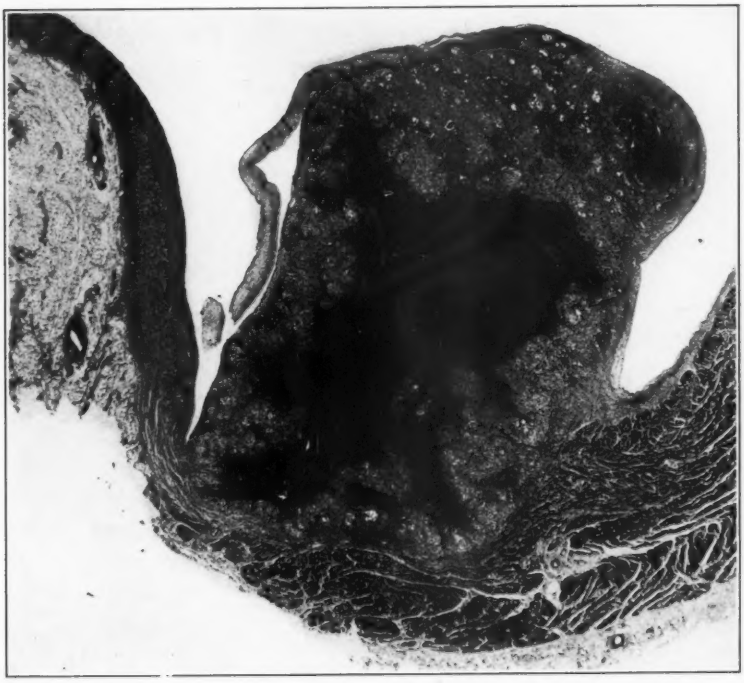
DESCRIPTION OF PLATE

PLATE 60

FIG. 1. Caseous tuberculous nodule at the base of the pulmonary valve. The distal portion of the cusp is folded over the pulmonary side of the lesion. × 32.

FIG. 2. Margin of the caseous area. Muscle fibers of the heart wall are shown in the lower left corner. × 160.



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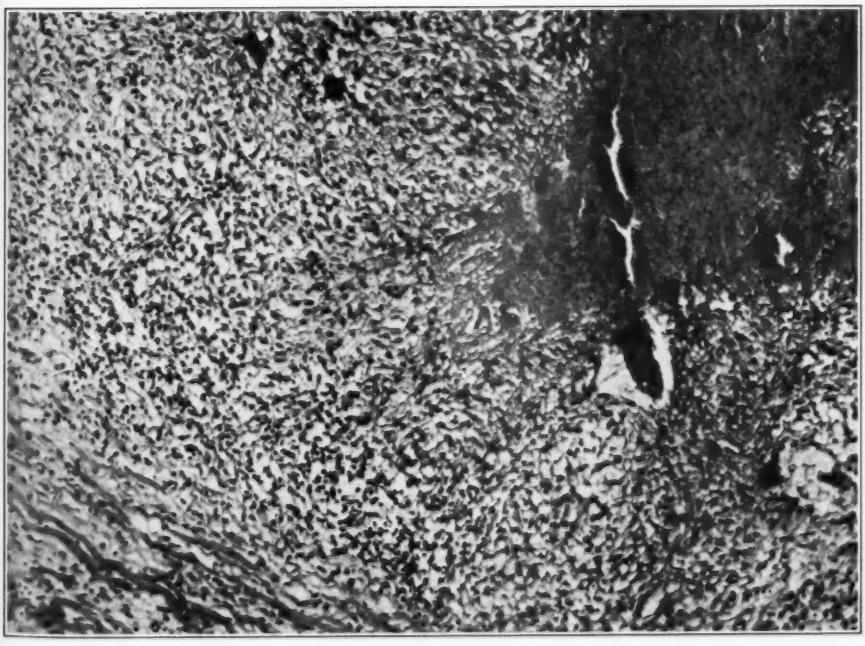

2

A SILVER CARBONATE STAINING METHOD FOR OLIGODENDROCYTES AND MICROGLIA FOR ROUTINE USE *

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The use of silver staining methods for oligodendrocytes and microglia is not widespread in pathological laboratories. Two of the reasons have been the necessity of special fixation and the variability of the results. A small addition to Penfield's [1] second modification of del Río Hortega's silver carbonate stain has given fairly uniform results on formalin-fixed central nervous system tissue. With attention to some details of time of fixation, as commented on below, successful and differential stains have been obtained quite regularly in our hands. The method used is simple and elastic, and lends itself to routine neuropathological use.

METHOD

1. The tissue is fixed by immersion in 10 per cent formalin.

2. Frozen sections are cut at 20 to 25 μ and are placed in distilled water, to which about 20 drops of strong ammonia are added for each 100 cc.

3. The sections remain in this dilute ammonia solution for a few minutes if recently fixed, to overnight for material that has been in formalin for several weeks.

4. Without washing, the sections are passed into Globus' [2] 10 per cent hydrobromic acid solution (10 cc. of 40 per cent hydrobromic acid to 90 cc. of distilled water), and are placed in the incubator at 37° C. for 1 hour.

5. The sections are then passed through 2 changes of distilled water.

6. Place the sections in 5 per cent sodium carbonate solution. After they are in the solution, approximately an equal quantity of a 5 per cent solution of ammonium alum (aluminum and ammonium sulphate) is added. This causes a white flocculent precipitate of aluminum hydroxide. The sections remain in this

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solution for 1 hour, although they may stay in this solution 2 to 3 days, if not convenient to stain sooner.

7. Wash well by passing through 2 changes of distilled water, gently opening out the sections, which will be found to be pliable and easily manipulated.

8. Stain in Hortega's strong silver carbonate solution until the sections turn a deep grayish brown on reduction, usually 2 to 5 minutes. (To 5 cc. of 10 per cent silver nitrate, add 20 cc. of 5 per cent sodium carbonate; then add ammonia, 28 per cent, drop by drop until the precipitate is just dissolved; filter and add 20 cc. of distilled water.)

9. Pass the sections directly to 1 per cent formalin, where they are agitated by blowing on them until reduction is complete.

10. Wash in distilled water.

11. Tone in a 1:500 yellow gold chloride solution, removing the sections just as soon as they are a uniform gray color.

12. Fix in a 5 per cent sodium thiosulphate solution about 3 to 5 minutes.

13. Wash in distilled water.

14. Tease sections onto a slide, blot with filter paper, dehydrate, clear, and mount in balsam.

There is a differential staining of oligodendrocytes and microglia. The oligodendrocytes are best obtained just at the time the tissue is well fixed in 10 per cent formalin, about 2 days fixation for spinal cord, and 4 to 5 days for the brain. Microglia are stained better after a somewhat longer fixation. There are no maximum or minimum times for fixation that can be given. However, the longer the tissue has been in formalin, the longer the sections should remain in the dilute ammonia water (Step 3).

The use of the sodium carbonate-ammonia alum mordant seems to aid in differential staining of the cytoplasm and processes of these cells. The other elements are a smooth gray in color and usually so lightly stained that they serve as an identifiable background in good contrast to the stained cells. The nuclei of the oligodendrocytes are not stained, and the processes may be stained a gray-black, which reveals details of structure such as gliosomes. The nuclei of the microglia take a light gray stain in contrast with the grayish black staining of the processes.

It should be noted that pathological alterations in these cells

render them more easily stained. Swollen oligodendrocytes, rod cells and compound granular corpuscles will stain well and smoothly after many weeks in formalin.

Summary

A modification of Penfield's method for silver staining of oligodendrocytes and microglia is reported. The method allows use of the formalin-fixed tissue and is easily applied. It has given good results in our hands, and it is believed this method should be of value in a neuropathological laboratory.

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DESCRIPTION OF PLATE

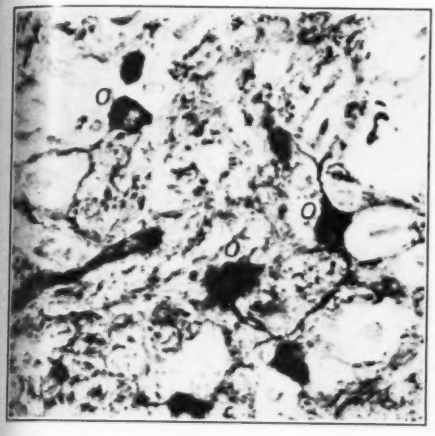
PLATE 61

FIG. 1. Normal oligodendrocytes "O" in a cross section of the spinal cord of a cat. Note the delicate processes winding about the myelin sheaths. Tissue fixed in 10 per cent formalin for 1 day.

FIG. 2. Normal oligodendrocytes in the medulla of a cat. One delicate process "P" is in focus for most of its length. Tissue fixed in 10 per cent formalin for 3 days.

FIG. 3. Normal microglia in the cerebral cortex of a monkey. A swollen oligodendrocyte is also seen. Tissue fixed in 10 per cent formalin for 2 weeks.

FIG. 4. Two rod cells "R" in a section of human cerebral cortex in general paresis. Several swollen oligodendrocytes "O" are also seen. Tissue fixed in 10 per cent formalin for 28 weeks.



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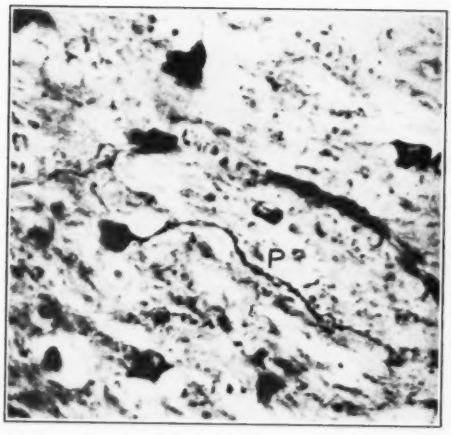

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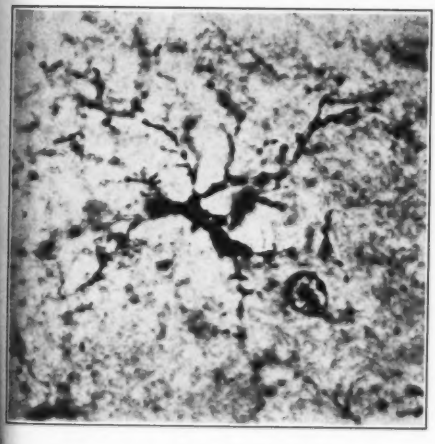

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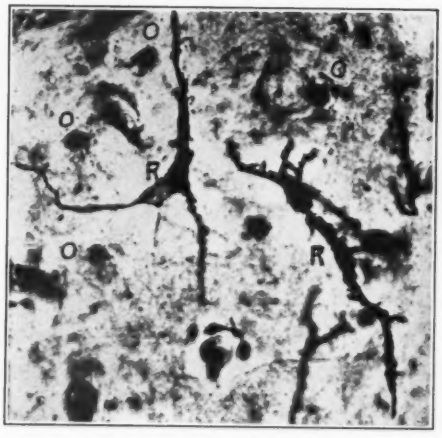

4

McCarter

Silver Carbonate Staining Method